Relationships between macroinvertebrate communities of stony littoral habitats and water chemistry variables indicative of acid-stress

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INTRODUCTION

Acidification is a serious threat to the biodiversity and functioning of Swedish inland surface waters, and it is estimated that some 14,000 or 15% of Swedish lakes with a surface area < 1 km^2 and about one-fifth of all watercourses can be regarded as being adversely affected by acidification (Bernes 1991). The short-term effects of acidification on aquatic communities are relatively well understood, with the most serious effects on taxon richness occurring at pH changes between 7 and 5.5 (Brodin 1995). Gastropods, bivalves, amphipods and mayflies (with the exception of *Leptophlebia* spp.) are generally considered as sensitive, whereas taxa such as water boatmen (Corixidae) and backswimmers (*Notonecta* spp.) and coleopterans (e.g. Dytiscidae) are more tolerant to acidification (Økland and Økland 1986). Hence, acidification often results in predictable changes in the balance between predator and prey organisms, with marked increases in certain large predatory insects such as dragonflies, water bugs, and beetles. In other words, as aquatic ecosystems become acidified the macroinvertebrate community shifts not only towards a predominance of predators, but collector-shredders also increase while scraper and collector-gatherer densities decline (Stokes et al. 1989).

Although the short-term effects of acidification on aquatic ecosystems are relatively well understood, knowledge is still lacking as to what chemical variables can be considered as drivers or proxies of the biological changes associated with acidification stress. Ideally, a biological variable is selected to directly monitor changes in ecosystem structure and function. However, although often preferable, the sole reliance on biological metrics in monitoring programs is not always justifiable for logistic and economic reasons. Generally the selection of a chemical metric as surrogate for a biological metric is build upon reliable cause and effect relationships. For example, total phosphorus concentrations are often used as a surrogate for phytoplankton in monitoring the effects of cultural nutrient enrichment on lake ecosystems.

The Swedish national environmental monitoring program consists of three nested tiers or levels of population and ecosystem resolution (e.g. Wiederholm and Johnson 1997). In the first tier, national lake and stream surveys are conducted (presently at five-year intervals) using physico-chemical and biological (macroinvertebrates) metrics to give an unbiased characterisation of the status ecosystem condition and show spatial or regional patterns in ecosystem status. To better understand the influence of interannual variability on selected metrics (i.e. those use in national surveys) and to aid in detecting trends in ecosystem degradation and recovery, a number of lakes (ca 100) and streams (ca 50) are monitored on an annual basis. For example, in the second tier physico-chemical metrics are monitored 4x annually, while macroinvertebrates and phytoplankton are monitored once annually (autumn and late summer sampling, respectively). In the third tier, a selected number of lakes (n = 15) and streams (n = 15) are monitored more intensively (higher within-year monitoring frequencies) to better understand the importance of interactions between physico-chemical and biological processes in the study sites as well as interactions between the sites and their catchments.

The large number of aquatic ecosystems in Sweden (e.g. > 100 000 lakes) constrains, for economic and logistic reasons, the sole use biological metrics in the national and to some extent even regional monitoring programs. Consequently, national lake and stream surveys as well as the large number of sites currently being limed to ameliorate the deleterious effects of acidification are monitored mainly using physico-chemical metrics. Clearly, if physico-chemical metrics are to be used as surrogates for biological metrics, then knowledge of dose-response relationships (as for total phosphorus and algal biomass) are a prerequisite for devising reliable conceptual models. Although a number of studies have focused on the effects of acidification on biological response variables (e.g. fish and macroinvertebrates), few studies have studied (in-depth) the empirical relationships. This study was designed to correlatively assess relationships between littoral macroinvertebrate communities and physico-chemical metrics (variables) indicative of acid stress. In particular, our primary goal was to determine what physico-chemical metrics are best correlated with changes in littoral macroinvertebrate composition and time frames are important (e.g. the importance of lag-phase responses). Building on this information of "dose-response" relationships we analyzed the data from a lake acidification gradient to determine if biological threshold(s) could be elucidated (extracted) between selected physico-chemical and biological metrics.

METHODS

Study lakes

In the national lake and stream data set (www.ma.slu.se) 126 lakes included both measures of water chemistry and littoral macroinvertebrates. However, to more unequivocally analyze the effects of acidity on macroinvertebrate communities, lakes judged to be affected by other anthropogenic stressors were removed from the data set. Consequently, lakes affected by agriculture (e.g. total phosphorus concentration > expected background levels or > 20 % of the catchment classified as agriculture), urbanization (> 0.1 % of the catchment classified as urban), and liming were removed from the data set resulting in 91 lakes distributed across the country (Fig. 1). A preliminary PCA ordination of lake water chemistry and catchment variables showed that one lake (Lake Bästetrask) had unusually high pH (pH = 8.2 compared with the population mean ± 1SD of 6.24 ± 0.72), hence this lake was also removed from the data set resulting in 90 lakes. Catchments were classified according to land use (Wilander et al., 2003).

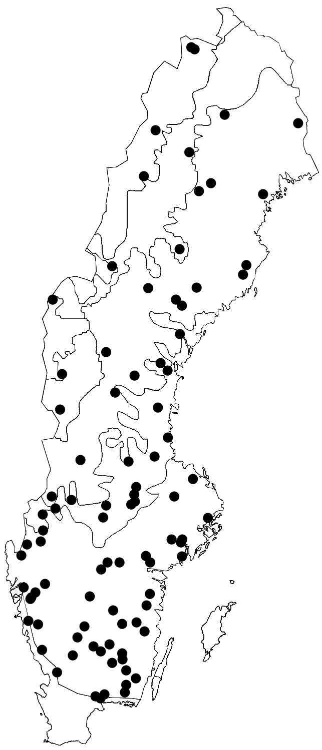

Figure 1. Location of the 90 "acid" reference lakes by six ecoregions.

Water chemistry

Surface water samples (0-2 m) were collected from mid lake using a plexiglas sampler. Samples were collected usually 4x annually (i.e. spring, summer, autumn and winter) All chemical analyses were done at the Department of Environmental Assessment and followed international (ISO) or European (EN) standards when available. Table 1 gives the variables measured and units. All variables with the exception of pH were either $\log_{10}$ or arc-sin square-root (proportional data) transformed in order to approximate normally distributed random errors. Hereafter, the physico-chemical variables are referred to collectively simply as water chemistry variables.

Table 1. Selected physico-chemical and land use variables.

Variables	Units
Geographic variables:	
X coordinates (≈ latitude)	
Y coordinates (≈ longitude)	
Catchment land use classification, etc:	
catchment area	km^2
annual temperature	^oC
annual precipitation	mm/year
annual runoff	mm/year
urban	percent
forest	percent
open	percent
alpine	percent
water	percent
mire	percent
agriculture	percent
lake area	km^2
lake altitude	m a.s.l.
mean depth	m
Water physico-chemical variables:*	
pH	
Alkalinity/Acidity	meq/L
ANC	meq/L
ANCalk	meq/L
ANCmod1	meq/L
ANCmod2	meq/L
ANCmod3	meq/L
ANCmod4	meq/L
ANCmod5	meq/L
ANC/H+	unitless
BC*/SSA	unitless
BC*/SO4	unitless
Aliminum	µg/L
Al3+	µg/L
H+Al3	µg/L
Ca/Ali	unitless
Ca	meq/L
Mg	meq/L
Na	meq/L
K	meq/L

SO4_IC	meq/L
Cl	meq/L
Al_s	µg/L
TOCc	mg/L
Temperature	^{o}C
Conductivity	(mS/m25)
NH4-N	µg/L
NO2+NO3-N	µg/L
Organic-N	µg/L
PO4-P	µg/L
Total phosphorus	µg/L
Water color	Abs._F 420/5
Si	mg/L
Fe	µg/L
Mn	µg/L
Secchi depth transparency	m
Chlorophyll a	mg/m3

** water variables were analyzed also at lags of 1and 2 years*

<u>Acid reference study lakes</u>
Table 2 lists the 90 lakes used here to assess the effects of acidification on littoral macroinvertebrate communities. On average, the lakes have been monitored for chemical and littoral macroinvertebrates for almost nine years; minimum study period was one year (n = 8) and the maximum study period was 16 years (n = 12). The majority of lakes were located in the mixed forest ecoregion (3) in the south (n = 48), followed by 32 lakes in the coniferous forest ecoregion and 10 lakes in the arctic/alpine ecoregion (Fig. 1).

Table 2. "Acid" reference lakes (n = 90). Ecoregion 1 = arctic/alpine region; ecoregion 2 = the boreal coniferous forest region and ecoregion 3 = the mixed forest region.

Name	Number of years	Ecoregion	X-coordinate (≈ latitude)	Y-coordinate (≈ longitude)
Abiskojaure	14	1	758208	161749
Dunnervattnet	7	1	713131	144608
Fjätsjön Övre	7	1	690617	134197
Latnjajaure	4	1	758677	161050
Louvvajaure	7	1	736804	160569
Njalakjaure	7	1	741340	153576
Stor-Arasjön	8	1	716717	158596
Stor-Björsjön	5	1	706083	132287
Stor-Tjulträsket	13	1	731799	151196
Ö. Särnamannasjön	2	1	683337	133785
Bjännsjön	9	2	713404	172465
Brännträsket	13	2	728095	175926
Dagarn	8	2	664197	149337
Degervattnet	6	2	708512	152086
Gipsjön	8	2	672729	138082

Gosjön	7	2	677506	156174
Gransjön	1	2	692866	154650
Hällsjön	7	2	667151	149602
Hällvattnet	8	2	704955	159090
Jutsajaure	15	2	744629	167999
Laxtjärnen	1	2	730329	165133
Limmingsjön	8	2	660804	142742
Långsjön	7	2	673534	153381
Mäsen	14	2	665654	149206
Pahajärvi	7	2	742829	183168
Remmarsjön	16	2	708619	162132
S. Bergsjön	1	2	706041	157858
Sangen	7	2	686849	145214
Spjutsjön	9	2	672467	148031
St. Gloppsjön	1	2	663308	143386
Stensjön	16	2	683673	154083
Stor-Backsjön	4	2	695220	143383
Tväringen	14	2	690345	149315
Täftesträsket	8	2	711365	171748
Ulvsjön	9	2	661521	130182
V. Rännöbodsjön	8	2	691365	156127
Valasjön	8	2	698918	158665
Vuolgamjaure	7	2	728744	162653
Ämten	1	2	665207	132083
Örvattnet	8	2	662682	132860
Översjön	8	2	664410	136192
Övre Skärsjön	16	2	663532	148571
Allgjuttern	16	3	642489	151724
Alsjön	9	3	647050	130644
Björken	8	3	652707	159032
Brunnsjön	16	3	627443	149526
Bysjön	16	3	658086	130264
Bäen	8	3	623624	141149
Djupa Holmsjön	8	3	656263	156963
Fagertärn	8	3	651558	143620
Fersjön	1	3	626033	147550
Fiolen	15	3	633025	142267
Fisjön	1	3	639293	127208
Fjärasjö	9	3	638725	146677
Fräcksjön	16	3	645289	128665
Försjön	4	3	641603	144848
Granvattnet	7	3	646293	126302
Grissjön	15	3	651578	146163
Gryten	7	3	652840	151589
Hagasjön	16	3	635878	137392
Harasjön	16	3	632231	136476
Hinnasjön	8	3	630605	144655
Hjärtsjön	8	3	632515	146675
Holmeshultasjön	5	3	634447	144024
Humsjön	15	3	650061	142276
Härsvatten	14	3	643914	127698
Hökesjön	8	3	639047	149701

Lilla Öresjön	8	3	638665	129243
Lillesjö	9	3	623161	142148
Mossjön	6	3	638085	138862
N. Yngern	9	3	656206	159170
Rotehogstjärnen	15	3	652902	125783
Siggeforasjön	7	3	665175	157559
Skärgölen	15	3	651573	152481
Skärsjön	9	3	633344	130068
St Skärsjön	16	3	628606	133205
St. Lummersjön	7	3	644463	139986
Stora Envättern	16	3	655587	158869
Stora Tresticklan	6	3	655209	126937
Storasjö	16	3	631360	146750
Sännen	14	3	624421	147234
Tomeshultagölen	7	3	629026	147562
Torrgårdsvattnet	1	3	644180	127892
Tängersjö	8	3	637121	151366
Tärnan	8	3	660688	164478
Vikasjön	2	3	668814	161417
Västra Solsjön	6	3	655863	129783
Älgarydssjön	15	3	633989	140731
Öjsjön	1	3	644987	152393
Örsjön	8	3	624038	143063

The "acid" reference lakes used in this study consisted predominantly of small (mean lake surface area = 1.1 km^2), nutrient poor (mean TP = 9.8 µg/L and chlorophyll a = 3.7 mg/m^3) ecosystems situated in forested catchments (Table 3). Exclusion of lakes affected by urbanization and agriculture resulted in a lake data set with pH ranging from 4.45 to 7.22 (10 percentile = 5.18 and 90 percentile = 6.92) and buffering capacity (alkalinity/acidity) ranging from –0.0615 meq/L to 0.5018 meq/L (10^{th} percentile = -0.0092 meq/L and 90^{th} percentile = 0.1833 meq/L) (Fig. 2). In organic aluminum concentrations averaged 39.3±84.5 µg/L, with concentrations ranging from 0.18 to 668 µg/L (10^{th} percentile = 2.01 and 90^{th} percentile = 86.1 µg/L). The ratio of base cations to the sum of strong acids ranged from 0.541 to 23.7 (10^{th} percentile = 1.13 and 90^{th} percentile = 6.26).

Table 3. Selected water chemical and land use/type and hydromorphological variables of 90 study lakes.

Variable	Mean ± 1SD
Altitude (m a.s.l.)	227 ± 194
Catchment area (km^2)	24 ± 56
Lake area (km^2)	1.1 ± 1.8
Mean depth (m)	5.1 ± 3.7
Percent urban	0.1 ± 0.7
Percent forest	73.6 ± 21.6
Percent open	1.7 ± 4.3
Percent alpine	5.2 ± 19.7
Percent water	12.7 ± 7.6

Percent mire	4.2 ± 8.8
Percent agriculture	1.5 ± 3.4
Temperature (°C)	4.5 ± 2.6
pH	6.2 ± 0.7
ANC (meq/L)	0.141 ± 0.122
TOCc (mg/L)	8.3 ± 4.4
Conductivity (mS/m25)	4.6 ± 2.2
NO2+NO3-N (µg/L)	55 ± 44.3
Org-N (µg/L)	352 ± 136
PO4-P (µg/L)	2.2 ± 0.7
Tot-P (µg/L)	9.8 ± 3.4
Water color (Abs._F 420/5)	0.117 ± 0.101
Transparency (m)	4.1 ± 2.5
Chlorophyll a (mg/m^3)	3.7 ± 2.4

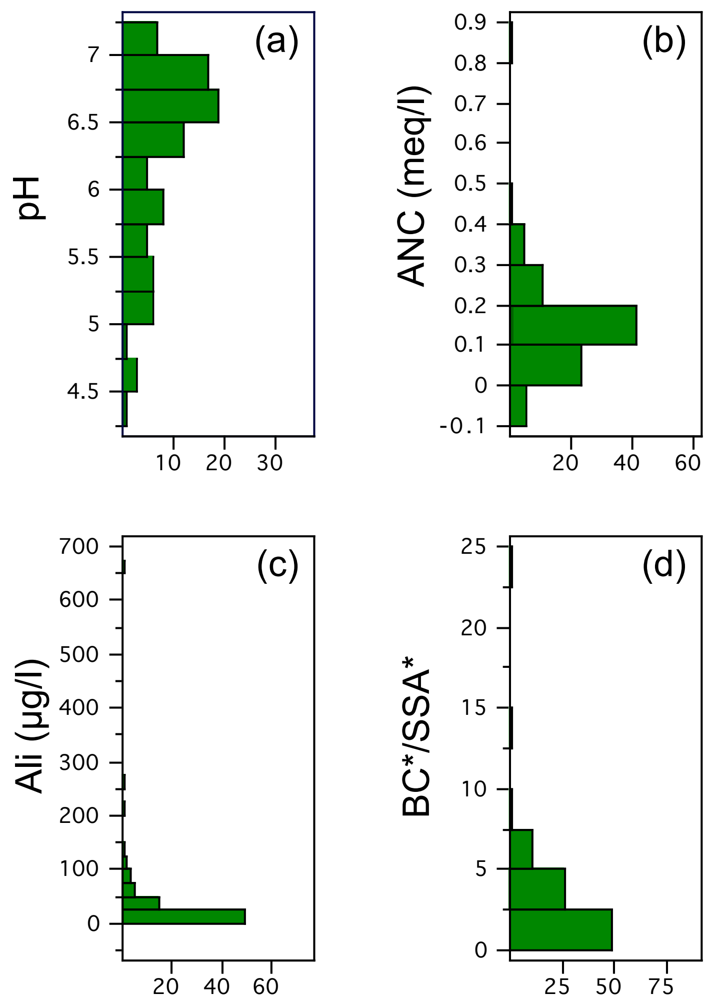

Figure 2. Distribution plots of selected acidification metrics.

Littoral macroinvertebrates

Littoral macroinvertebrate samples were collected in autumn (September to November) from stony habitats (wind exposed littoral regions) using standardized kick-sampling with a handnet (European Committee for Standardisation 1994) and mesh size of 0.5 mm and preserved in 70 % ethanol. In the laboratory, samples were processed by sorting under 10x magnification against a black background, followed by identification using dissecting and light microscopy. Organisms were identified to the lowest taxonomic unit possible, generally to the species level, although exceptions occurred with some chironomid larvae and immature oligochaetes. Only taxa that occur in > 1% of the lakes were used here (i.e. the species by site data set consisted of 326 taxa).

Statistical analyses

Constrained ordination

Direct gradient analysis (also known as constrained ordination, ter Braak, 1988, 1990) was used to select environmental variables that could explain significant amounts of the variability in structural composition among the littoral macroinvertebrate communities. Detrended correspondence analysis (DCA) of square-root transformed species abundance, with downweighting of rare taxa, detrending by segments and non-linear rescaling was used to determine the biological turnover, or gradient length, of the species dataset. From this the appropriate model (ordination procedure) for the constrained ordination was chosen. DCA of the taxonomic composition of the 90 lakes gave gradient lengths of 3.335 for axis 1 and 2.586 for axis 2, indicating that a unimodal response would adequately fit the species data. In CCA the species abundance data were square-root transformed and, where necessary, the environmental variables were transformed ($\log_{10}$ or arcsine of square root) in order to approximate normally distributed random errors. Constrained ordinations were run using the species downweighting option and forward selection of environmental variables. Significance of the environmental variables was tested with 999 Monte Carlo permutations. TWINSPAN analysis (Hill, 1979) was used to select taxa indicative of the variability among community types. Only taxa selected as strong preferential taxa in the first three divisions of TWINSPAN are shown in the CCA ordination of community composition.

Partial constrained ordination

The total variation in an ecological data set can be partitioned into: (i) unique or pure variation from a specific variable, (ii) common variation contributed by all measured variables and (iii) random error (Hopke, 1992). Constrained ordination, as used above, does not explicitly test for the unique effect of the categories of spatial scale on taxonomic and functional composition. A number of techniques have been developed recently, however, for analyzing and partitioning the variance of multi-scale studies (e.g. Gustafson, 1998). The approach used here is based on constrained ordination (ter Braak, 1988); partial constrained ordination is used to explore the relationships between single environmental variables and biological response variables (e.g. Borcard, Legendre and Drapeau*,* 1992). Here, partial constrained ordination (pCCA) was run to remove the effect of year or geographic position and land use. pCCA of littoral macroinvertebrate assembalges (year = 2000) and land use and chemical variables (mean, median and extreme values) for t_0, (same year measures) and t_1 (measures with a one year lag).

A number of ordinations were done to analyze the importance of water chemical metrics on littoral macroinvertebrate community structure. First, correspondence analysis of macroinvertebrates and principle components analysis were used to examine the data sets. Canonical correspondence analysis of macroinvertebrates, geographic position, land use/type, lake hydromorphological descriptors and water chemistry was used to assess the importance of mean, extreme (minimum and maximum within-year values) and lag-phase (one and two year lags) responses on littoral communities. Partial correspondence analysis (pCCA) was run on macroinvertebrates and water chemistry with year run as a covariable (model 1). To partly remove the influence of geographic position, three pCCAs were run on macroinvertebrate communities, geographic position, land use/type, lake hydromorphological variables and water chemistry for the three ecoregions (models 2 to 4). Finally, to analyze the influence of water chemistry on macroinvertebrate communities all variables with the exception of water chemistry were run as covariables (model 5).

RESULTS AND DISCUSSION

Correspondence analysis

Figure 3 shows the results of a correspondence analysis of littoral macroinvertebrate assemblages. Eigenvalues of the first four axes were 0.181, 0.157, 0.088 and 0.069, respectively. Cumulatively, the first four axes explained 34.2% of the variance in the species data set; axis explained 12.5%, axis 2 explained 10.9%, axis 3 explained 6.1% and axis 4 explained 4.7%. TWINSPAN showed two indicator taxa for the first division, the chironomid midge *Endochironomus* sp. and *Asellus aquaticus* and the stonefly *Capnia atra* and the mayfy *Ameletus inopinatus*. *Capnia atra*, restricted for the most part to the arctic/alpine region in the north indicates nutrient poor conditions, while *Endochironomus* indicate more nutrient rich lakes in the southern part of the country.

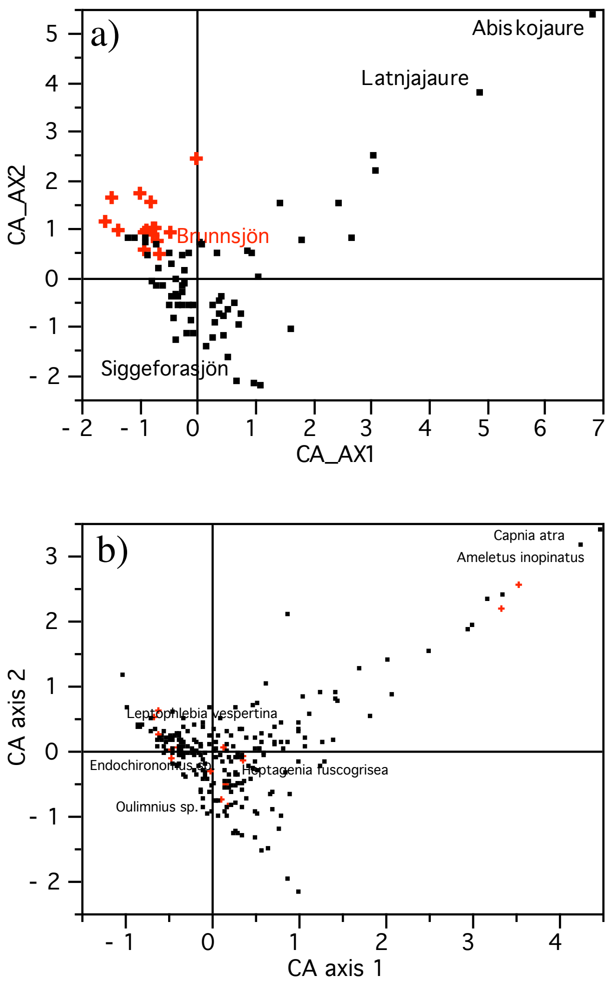

Figure 3. Correspondence analysis (CA) of littoral macroinvertebrates (a) and sites (b) from 90 "acid" reference lakes.

Principle components analysis

Principle components analysis of catchment land use and mean water chemical variables for the 90 lakes showed three important environmental gradients (eigenvalues were 17.2, 13.0 and 6.9, for PC axes 1 to 3, respectively). The first PC axis (PC1) accounted for 31.9% of variance and was strongly related to water chemistry variables indicative of acidification/acidity (Table 4). The second PC axis (PC2) explained another 24.1% of the variability among lakes and can interpreted as a productivity gradient. For example, chlorophyll a and total phosphorus were positively correlated with this axis, while altitude and latitude (x coordinates) were negatively associated with this axis (Fig. 4). The third PC axis (PC3) explained 12.7% of the remaining variance. Water column variables indicative of brown water systems (e.g. water color measured as absorbance of filtered water and Secchi depth transparency) and land use classification as mire were important descriptors of this axis. Cumulatively, these three environmental gradients: pH, latitude/productivity and water color, explained 68.7% of the variability among the 90 lakes studied here.

Table 4. PCA loadings (eigenvectors) for the first three axes of PCA analysis of water chemical, land use/type and hydromorphological variables for 90 "acid" reference lakes. Loadings ≥ 0.20 are marked in bold.

	PCA 1	***PCA 2***	***PCA 3***
Eigenvalue	17.2	13.0	6.9
Percent	31.9	24.1	12.7
Geographic variables:			
X coordinate (≈ latitude)	0.117	-0.188	0.139
Y coordinate (≈ longitude)	0.139	-0.017	0.108
Catchment land use, etc.			
Catchment area	0.124	-0.081	0.088
Annual Temperature			
Annual Precipitation	-0.119	-0.087	-0.073
Annual Runoff	-0.032	-0.162	0.007
Urban	0.030	0.056	-0.017
Forest	-0.021	0.148	0.043
Open	0.006	0.045	-0.056
Alpine	0.038	-0.184	-0.034
Water	-0.031	-0.025	**-0.237**
Mire	0.051	-0.011	**0.245**
Agriculture	0.003	0.105	-0.144
Lake area	0.115	-0.105	-0.038
Lake altitude	-0.025	**-0.215**	0.103
Depth	0.083	-0.106	-0.160
Water physico-chemical variables:			
pH	**0.223**	0.000	-0.059
Alkalinity/Acidity	**0.227**	0.049	-0.016
ANC	**0.210**	0.110	0.041
ANCalk	**0.213**	0.101	0.043
ANCmodel 1	**0.225**	0.058	-0.006
ANCmodel 2	**0.224**	0.067	0.004

ANCmodel 3	**0.222**	0.076	0.013
ANCmodel 4	**0.219**	0.084	0.023
ANCmodel 5	**0.217**	0.092	0.031
ANC/H+	**0.229**	0.040	-0.037
BC*/SSA*	0.198	-0.005	0.189
BC*/SO4*	0.196	-0.006	0.192
Ali	**-0.212**	0.024	0.074
Al3+	**-0.220**	0.012	0.065
H+Al3	**-0.220**	0.012	0.065
Ca/Ali	**0.219**	0.053	-0.100
Ca	0.158	0.172	-0.075
Mg	0.036	**0.220**	-0.156
Na	-0.069	**0.215**	-0.160
K	0.035	0.176	-0.180
SO4_IC	-0.060	0.178	**-0.240**
Cl	-0.091	0.190	-0.188
Al_s	-0.171	0.083	0.140
TOCc	0.004	**0.217**	0.188
Water temperature	-0.074	0.172	-0.070
Conductivity	0.008	**0.217**	-0.191
NH4-N	-0.160	0.090	0.017
NO2+NO3-N	-0.103	0.121	-0.114
Organic-N	0.020	**0.242**	0.065
PO4-P	-0.058	0.157	0.197
Total phosphorus	-0.021	**0.211**	0.138
Water color	-0.023	0.172	**0.274**
Si	0.018	0.097	**0.261**
Fe	-0.084	0.139	**0.271**
Mn	-0.133	0.153	0.111
Secchi depth transparency	0.054	-0.160	**-0.269**
Chlorophyll a	-0.041	**0.226**	0.048

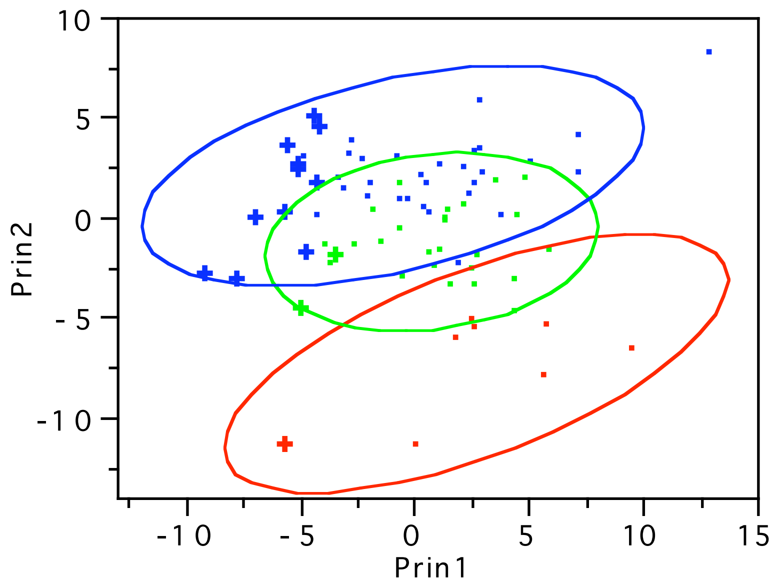

Figure 4. Scatter plot of principle component axes 1 (Prin 1) and 2 (Prin 2). Variables in the PCA consisted of land use and water column chemical variables of 90 lakes. Eclipses show ecoregion delineation; red = placement in the arctic/alpine, green = placement in the coniferous forest, and blue = placement in the mixed forest ecoregion. + show lakes that have mean pH ≤ 5.5.

<u>CCA of littoral assemblages and mean and extreme chemical variables</u>

Constrained ordination of littoral macroinvertebrates and geographic position, land use/type and hyrdromorphological variables showed that six of the 15 variables individually explained > 10% of the variance among lakes (Table 5). Geographic position, in particular latitude, as well as variables indicative of ecosystem size (e.g. catchment area) and land use/type were important descriptors communities. Catchments classified as alpine explained 24% of the variance among lakes. The influence of alpine catchments and latitude is not surprising given the broad climatic gradient from the southern to northern parts of the country. In other words, both of these variables might be interpreted as proxies of temperature and, indirectly, ecosystem productivity. Variables of ecosystem size (e.g. lake area, 17% and catchment area, 12%) and lake altitude and depth (each explained 11%) also explained > 10% of the among-lake variance in macroivertebrate assemblages.

Table 5. Canonical correspondence analysis of littoral macroinvetebrate communities of ”acid” reference lakes and geographic, land use/type and hydromorphological variables. Variables explaining > 10% of the among-lake variance are shown in bold.

Variable	λ1	p-value
X coordinate (≈ latitude)	**0.13**	***
Y coordinate (≈ longitude)	0.06	*
Catchment area	**0.12**	***

Annual Precipitation	0.05	0.177
Annual Runoff	0.07	*
Urban	0.03	0.719
Forest	0.08	**
Open	0.06	0.098
Alpine	**0.24**	***
Water	0.05	0.19
Mire	0.07	*
Agriculture	0.05	0.167
Lake area	**0.17**	***
Lake altitude	**0.11**	***
Depth	**0.11**	***

The importance of annual means versus within-year extreme values (i.e. observed minimum and maximum values) as well as the potential importance of lag-phase responses was studied using a two-year subset of "acid" reference lake data set. Data for the year 2000 (for chemistry and macroinvertebrates) and 1999 (for assessing the importance of one-year lag in water chemical variables on macroinvertebrates) were selected and analyzed using canonical correspondence analysis. Although not analyzed here, the year 2000 was selected since a larger data set (data from ca 700 lakes) is available from the national lake survey for validating these results.

Several variables indicative of acid stress (e.g. pH and inorganic Al) explained > 10% of the among-lake variance in littoral macroinvertebrate assemblages, while variables indicating other forms of stress (e.g. total phosphorus as an indicator of eutrophication) were seemingly of less importance (Table 6). Somewhat unexpected was the finding that variables indicating buffering capacity (e.g. alkalinity and ANC) were of less importance than pH or Al fractions. One exception was the ratio of ANC/H^+; this variable explained 11% of the variance in littoral assemblages. The importance of chemical measures one year before macroinvertebrates were sampled (i.e. t_1) was equivocal. For example, 12 variables explained > 10% of the variance using values at t_0 and 13 variables at t_1. The importance of inorganic nitrogen (NO_2+NO_3-N) increased from 7 to 11%, when t_0 and t_1 were compared. Perusal of extreme values showed that 10 variables (for both t_0 and t_1) explained > 10% of the among-lake variance. Maximum chlorophyll a (a proxy for ecosystem production) explained 13% at t_0 and 11% at t_1 of the variance.

Table 6. Canonical correspondence analysis of littoral macroinvertebrate communities of lakes along an acid gradient and mean and extreme, min or max, values of selected chemical variables. Lakes were sampled in 1999 (for lag 1-year chemistry) and 2000. Lambda 1 (λ1) shows the variance explained without running covariables in the constrained ordination. Variables explaining > 10% of the among-lake variance are shown in bold.

Variable	Annual mean value				Within -year extreme value			
	Mean		Mean with 1 lag year		Extreme		Extreme with 1 lag year	
	λ1	p-value	λ1	p-value	λ1	p-value	λ1	p-value
pH	**0.13**	***	**0.12**	***	**0.11**	***	0.09	**
Alkalinity/Acidity	0.07	0.055	0.07	*	0.07	*	0.05	0.185
ANC	0.06	0.057	0.06	0.08	0.05	0.218	0.05	0.132
ANCalk	0.06	0.073	0.06	0.059	0.05	0.119	0.05	0.222
ANCmodel 1	0.07	0.055	0.08	*	0.06	0.054	0.06	*
ANCmodel 2	0.07	*	0.08	**	0.06	0.08	0.06	0.067
ANCmodel 3	0.07	0.053	0.07	*	0.06	0.078	0.06	0.088
ANCmodel 4	0.06	0.066	0.07	*	0.06	0.089	0.05	0.138
ANCmodel 5	0.06	0.077	0.06	*	0.06	0.108	0.05	0.158
ANC/H+	**0.11**	***	0.1	***	**0.1**	***	0.08	**
BC*/SSA*	0.08	*	0.08	*	**0.1**	***	0.08	*
BC*/SO4*	0.08	*	0.08	*	**0.1**	***	0.08	*
Ali	**0.1**	***	**0.1**	***	0.08	***	0.09	***
Al3+	**0.11**	***	**0.11**	***	0.09	***	**0.1**	***
H+Al3	**0.11**	***	**0.11**	***	0.09	***	**0.1**	***
Ca/Ali	**0.11**	***	**0.11**	***	0.08	**	0.09	***
Ca	0.08	**	0.08	***	0.08	**	0.09	***
Mg	**0.1**	***	**0.11**	***	**0.11**	***	**0.11**	***
Na	**0.11**	***	**0.13**	***	**0.12**	***	**0.13**	***
K	0.08	***	0.09	***	0.09	***	**0.10**	***
SO4_IC	**0.11**	***	**0.12**	***	**0.13**	***	**0.12**	***
Cl	**0.11**	***	**0.12**	***	**0.12**	***	**0.12**	***
Al_s	0.09	***	**0.1**	***	0.08	*	0.07	**

TOCc	0.08	***	**0.11**	***	0.07	*	0.07	*
Water temperature	0.09	***	0.04	0.476	0.05	0.298	0.05	0.383
Conductivity	**0.11**	***	**0.12**	***	**0.12**	***	**0.13**	***
NH4-N	**0.1**	***	0.08	***	0.06	*	0.04	0.465
NO2+NO3-N	0.07	**	**0.11**	***	0.08	**	**0.13**	***
Organic-N	0.06	0.093	0.07	*	0.06	0.056	0.05	0.239
PO4-P	0.06	0.082	0.07	*	0.05	0.167	0.02	0.964
Total phosphorus	0.09	***	0.09	***	0.08	***	0.06	*
Water color	0.08	**	0.09	***	0.09	**	0.07	**
Si	0.05	0.146	0.07	*	0.05	0.109	0.05	0.402
Fe	0.08	***	0.09	***	0.09	*	0.08	**
Mn	0.08	***	0.09	***	0.08	*	0.10	***
Secchi depth transparency	0.06	*		•	•		•	
Chlorophyll a	0.08	***	0.09	**	**0.13**	***	**0.10**	***
• missing data; p < 0.05 *; p < 0.01 **; p < 0.001***								

CCA of littoral assemblages and mean and extreme chemical variables (model 1)
Constrained ordination of littoral macroinvertebrate assemblages was analyzed using a data set consisting of geographic position, land use/type, hydromorphological and selected chemical variables (annual mean and within-year extreme values). Using stepwise forward selection, 13 variables explained 41% of the total variance in littoral macroinvertebrate assemblages among the “acid” reference lakes (Table 7). The first three variables selected (alpine catchments, max SO_4 and lake area) explained 24% of the total explained variance. Figure 5 shows the distribution of the lake sample scores and significant environmental variables. Alpine catchments was the first variable selected and explained 11% of the variance; this variable was positively correlated with lake altitude (which explained another 3% of the variance) and X coordinates (2%) and negatively correlated with max SO_4 (7%). These variables basically indicate the importance of a latitudinal (or productivity) gradient in the ordination of macroinvertebrate assemblages, with lakes in the upper right hand corner of the figure consisting of nutrient poor ecosystems (e.g. Lake Abiskojaure) in the north and more nutrient rich a ecosystems in the south (lower left hand corner of the ordination) and lakes with low buffering capacity and pH in the south (upper left hand corner of the ordination).

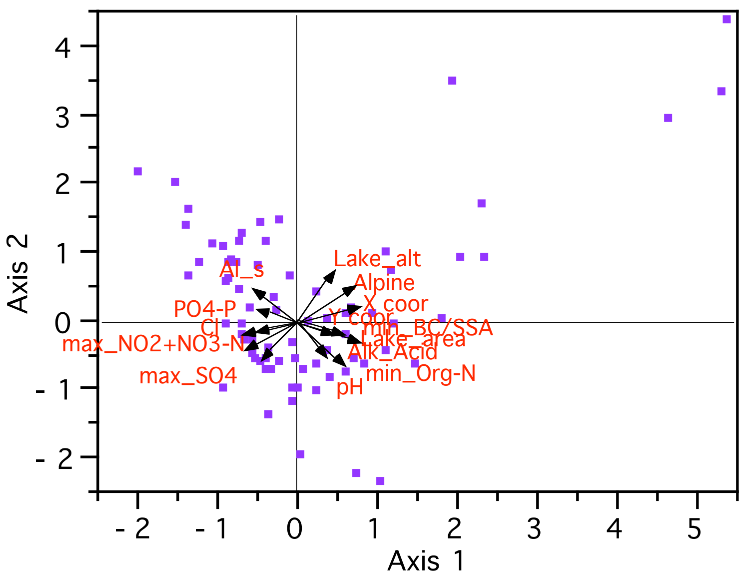

Figure 5. CCA biplot of “acid” reference lakes and environmental variables.

Table 7. Canonical correspondence analysis (CCA) of littoral macroinvertebrates and environmental variables (water chemical variables included mean and extreme, min or max, values). All variables selected explained significant amounts of among-lake variability ($p < 0.001$). Stepwise regression was stopped when no remaining variables explained > 1% of the remaining variance. The first 10 steps are shown in bold text.

Variable	λ1	model	step
X coordinate (≈ latitude)	0.11	0.02	13
Y coordinate (≈ longitude)	0.05	0.02	11
Catchment area	0.08		
Lake altitude	**0.09**	**0.03**	**5**
Lake area	**0.12**	**0.06**	**3**
Depth	0.06		
Forest	0.03		
Open	0.02		
Alpine	**0.11**	**0.11**	**1**
Water	0.04		
Mire	0.04		
Agriculture	0.03		
pH	0.12		
Alkalinity/Acidity	**0.08**	**0.03**	**4**
ANC	0.07		
ANCalk	0.06		
ANC/H+	0.11		
BC*/SO4*	0.08		
Ali	0.09		
Al3+	0.1		
H+Al3	0.1		
Ca/Ali	0.11		
SO4_IC	0.09		
Cl	0.09	0.02	12
Al_s	**0.08**	**0.02**	**9**
TOCc	0.05		
Water temperature	0.07		
Conductivity	0.09		
NO2+NO3-N	0.06		
Organic-N	0.07		
PO4-P	**0.05**	**0.03**	**6**
Total phosphorus	0.05		
Water color	0.04		
Chlorophyll a	0.07		
min pH	0.12		
min Alkalinity/Acidity	0.08		
min ANC	0.07		
min ANCalk	0.07		
minANC/H+	0.1		
min BC*/SSA*	**0.09**	**0.03**	**8**
min BC*/SO4*	0.09		
max Ali	0.08		

max Al3+	0.1		
max H+Al3	0.1		
min Ca/Ali	0.1		
max SO4_IC	**0.09**	**0.07**	**2**
max Cl'	0.09		
max Al_s	0.08		
max TOC	0.05		
min Water temp	0.05		
min Conductivity	0.09		
max NO2+NO3-N	**0.06**	**0.02**	**10**
min Org-N	**0.06**	**0.03**	**7**
min PO4-P	0.05		
min Tot-P	0.05		
max water color	0.05		
min Chlorophyll a	0.09		
total inertia		*1.446*	
Sum of all unconstrained eigenvalues (after fitting covariables)		*1.446*	
Sum of all canonical eigenvalues (after fitting covariables)		*0.593*	

<u>pCCA of littoral assemblages and mean and extreme water chemical variables by ecoregion and running geographic position and land use/type as covariables (models 2 – 4)</u>

Separate constrained ordinations of littoral macroinvertebrate assemblages and geographic position, land use/type and water chemical variables were run for the three major ecoregions in Sweden. Namely, the arctic/alpine ecoregion in the north, the boreal, coniferous ecoregion (consisting of the northern, middle and southern boreal regions) in the upper and central parts of the country and the mixed forest ecoregion (consisting of the boreonemoral and nemoral regions) in the south. Seven years of annual monitoring data were used in this analysis with time (year) run as a covariable.

<u>The arctic/alpine ecoregion (model 2)</u>
Table 8 shows the results of the partial CCA of lakes situated in the arctic/alpine ecoregion. Twelve variables explained 39% of the among-lake variance after accounting for the effects of geographic position and land use/type. Alpine catchments was the first variable selected and explained 18% of the variance. This indicates that although the lakes are situated in the arctic/alpine ecoregion, that a gradient in alpine catchment type still exists among the lakes. In contrast to the CCA ordination of whole data set, several land use/types explained significant amounts of variance. For example, besides alpine catchments, catchments characterized as open (9%), agriculture (9%) and the amount of water or the number of other waterbodies (7%) all were important predictors of assemblages. Not surprisingly, variables indicative of acidification did not explain significant amounts of the variance among lakes in this ecoregion.

Table 8. Partial canonical correspondence analysis (pCCA) of littoral macroinvertebrates and environmental variables (water chemical variables included mean and extreme, min or max, values) in the arctic/alpine ecoregion (ecoregion 1). Data from the years 1999 to 2002 were included in the analyses, with year run as a covariable. All variables selected explained significant amounts of among-lake variability ($p < 0.05$). Stepwise regression was stopped when no remaining variables explained > 1% of the remaining variance. The first 10 steps are shown in bold text.

Variable	$\lambda 1$	model	step
X coordinate (≈ latitude)	**0.17**	**0.15**	**2**
Y coordinate (≈ longitude)	0.15		
Annual Precipitation	0.12		
Annual Runoff	0.1		
Catchment altitude	0.17		
Catchment area	**0.13**	**0.12**	**3**
Lake altitude	**0.12**	**0.07**	**7**
Lake area	**0.12**	**0.06**	**8**
Depth	0.12		
Urban	omitted		
Forest	0.16		
Open	**0.16**	**0.09**	**5**
Alpine	**0.18**	**0.18**	**1**
Water	**0.14**	**0.07**	**6**
Mire	0.11		
Agriculture	**0.1**	**0.09**	**4**
pH	0.08		
Alkalinity/Acidity	0.07		
ANC	0.07		
ANCalk	0.07		
ANCmodel 1	0.07		
ANCmodel 2	0.07		
ANCmodel 3	0.07		
ANCmodel 4	0.07		
ANCmodel 5	0.07		
ANC/H+	0.08		
BC*/SSA*	0.11		
BC*/SO4*	0.11		
Ali	missing		
Al3+	missing		
H+Al3	missing		
Ca/Ali	missing		
Ca	**0.09**	**0.06**	**10**
Mg	0.11		
Na	0.09		
K	0.11		
SO4_IC	0.11		
Cl	0.07		
Al_s	0.07	0.04	12
TOCc	0.13	0.05	11
Water temperature	0.12		
Conductivity	0.08		
NO2+NO3-N	0.06		
Organic-N	0.07		

PO4-P	0.07		
Total phosphorus	**0.07**	**0.06**	**9**
Water color	0.14		
Si	0.1		
Fe	0.1		
Mn	0.08		
total inertia		*2.657*	
Sum of all unconstrained eigenvalues (after fitting covariables)		*2.586*	
Sum of all canonical eigenvalues (after fitting covariables)		*1.016*	

<u>The boreal coniferous ecoregion (model 3)</u>
Similar to the arctic/alpine ecoregion, one of the most important variables explaining the among-lake variance in macroinvertebrate assemblages was the landscape variable the percentage of a catchment classified as mire (Table 9). Although lake area was selected in the first step of CCA, this variable and percent catchment mire were selected in the first two steps and explained 8% and 7%, respectively, of the total variance. Altogether, 20 variables were selected, and cumulatively these variables accounted for 35.7% of the total variance. Other significant predictors of macroinvertebrate assemblages in the coniferous forest ecoregion were lake altitude (5%, selected in step 3), Y coordinates (≈ longitude, 5%, step 4), catchment area (4%, step 5) and annual precipitation (5%, step 6). Several of these variables, together with the importance of alpine catchments (3%, step 8), seemingly indicate the importance of a west to east (or longitudinal) gradient, from the alpine areas in the east with high precipitation to the lowland, somewhat dryer coastal regions in the west.

Table 9. Partial canonical correspondence analysis (pCCA) of littoral macroinvertebrates and environmental variables (water chemical variables included mean and extreme, min or max, values) in the coniferous forest ecoregion (ecoregion 2). Data from the years 1999 to 2002 were included in the analyses, with year run as a covariable. All variables selected explained significant amounts of among-lake variability ($p < 0.001$). Stepwise regression was stopped when no remaining variables explained > 1% of the remaining variance.

Variable	$\lambda 1$	model	step
X coordinate (≈ latitude)	**0.08**	**0.02**	**10**
Y coordinate (≈ longitude)	**0.06**	**0.05**	**4**
Annual Precipitation	**0.05**	**0.04**	**6**
Annual Runoff	0.03	0.02	20
Catchment altitude	**0.06**	**0.03**	**9**
Catchment area	**0.06**	**0.04**	**5**
Lake altitude	**0.07**	**0.05**	**3**
Lake area	**0.08**	**0.08**	**1**
Depth	0.04	0.02	11
Urban	0.04	0.02	15

Forest	0.04		
Open	0.04	0.02	16
Alpine	**0.05**	**0.03**	**8**
Water	0.04	0.02	12
Mire	**0.08**	**0.07**	**2**
Agriculture	0.04	0.02	17
pH	0.05	0.02	13
Alkalinity/Acidity	0.04		
ANC	0.04		
ANCalk	0.04		
ANCmodel 1	0.04		
ANCmodel 2	0.04		
ANCmodel 3	0.04		
ANCmodel 4	0.04		
ANCmodel 5	0.04		
ANC/H+	0.05		
BC*/SSA*	0.06		
BC*/SO4*	0.06	0.02	19
Ali	0.04		
Al3+	0.04		
H+Al3	0.04		
Ca/Ali	0.04		
Ca	0.05		
Mg	**0.05**	**0.03**	**7**
Na	0.06	0.02	18
K	0.05		
SO4_IC	0.08		
Cl	0.06	0.02	14
Al_s	0.04		
TOCc	0.04		
Water temperature	0.02		
Conductivity	0.06		
NO2+NO3-N	0.05		
Organic-N	0.02		
PO4-P	0.02		
Total phosphorus	0.03		
Water color	0.04		
Si	0.02		
Fe	0.04		
Mn	0.03		
total inertia		*1.841*	
Sum of all unconstrained eigenvalues (after fitting covariables)		*1.805*	
Sum of all canonical eigenvalues (after fitting covariables)		*0.644*	

The mixed forest ecoregion (model 4)

In contrast to CCA ordinations in the arctic/alpine and coniferous forest ecoregions where the importance of acidification variables for explaining among-lake variance in macroinvertebrate assemblages was negligible, several variables indicative of acidification stress were important descriptors in the mixed forest ecoregion of southern Sweden (Table 10). Lake pH was the first variable selected, and this variable alone accounted for 15% of the among-lake variance in littoral assemblages. Likewise, two other acidification variables were selected in the first ten variables selected; namely, ANCalk, (3%, step 4) and alkalinity/acidity (2%, step 7). In total, 15 variables were selected and accounted for 25.1% of the total variance. The second variable selected was lake area (7%), followed by catchment altitude (3%). Two landscape variables, percent forest (2%) and percent mire (2%) were also significant. These latter two variables presumably indicate the diverse landscape of this ecoregion, and the importance of forested catchments nested in a predominantly agricultural landscape.

Table 10. Partial canonical correspondence analysis (pCCA) of littoral macroinvertebrates and environmental variables (water chemical variables included mean and extreme, min or max, values) in the mixed forest ecoregion (ecoregion 3). Data from the years 1999 to 2002 were included in the analyses, with year run as a covariable. All variables selected explained significant amounts of among-lake variability ($p < 0.001$). Stepwise regression was stopped when no remaining variables explained > 1% of the remaining variance.

Variable	λ1	model	step
X coordinate (≈ latitude)	0.03	0.02	11
Y coordinate (≈ longitude)	**0.04**	**0.02**	**10**
Annual Temperature	0.03	0.02	15
Annual Precipitation	**0.03**	**0.02**	**8**
Annual Runoff	0.03	0.02	13
Catchment altitude	**0.08**	**0.03**	**3**
Catchment area	0.07		
Lake altitude	0.08		
Lake area	**0.12**	**0.07**	**2**
Depth	0.08	0.02	14
Urban	0.03		
Forest	**0.03**	**0.02**	**9**
Open	0.04		
Alpine	omitted, low s^2		
Water	0.07		
Mire	**0.03**	**0.02**	**6**
Agriculture	0.03		
pH	**0.15**	**0.15**	**1**
Alkalinity/Acidity	**0.12**	**0.02**	**7**
ANC	0.1		
ANCalk	**0.09**	**0.03**	**4**
ANCmodel 1	0.11		
ANCmodel 2	0.11		
ANCmodel 3	0.11		
ANCmodel 4	0.1		
ANCmodel 5	0.1		

ANC/H+	0.15		
BC*/SSA*	0.05		
BC*/SO4*	0.05		
Ali	0.11		
Al3+	0.12		
H+Al3	0.12		
Ca/Ali	0.14		
Ca	0.11		
Mg	0.08		
Na	**0.03**	**0.02**	**5**
K	0.05		
SO4_IC	0.05		
Cl	0.02		
Al_s	0.1		
TOCc	0.04		
Water temperature	0.01		
Conductivity	0.07		
NH4-N	0.06		
NO2+NO3-N	0.02	0.02	12
Organic-N	0.02		
PO4-P	0.02		
Total phosphorus	0.02		
Water color	0.08		
Si	0.05		
Fe	0.09		
Mn	0.05		
total inertia		*1.907*	
Sum of all unconstrained eigenvalues (after fitting covariables)		*1.87*	
Sum of all canonical eigenvalues (after fitting covariables)		*0.469*	

pCCA with land use/type and geographic position as covariable (model 5)

To more unequivocally analyze the effects of water chemical, and in particular variables indicative of acidification, on littoral macroinvertebrate assemblages of "acid" reference lakes, non-water chemical variables (e.g. geographic position, land use/type, ecosystem size) were run as covariables in the three constrained ordinations.

Nine variables were selected in the constrained ordination of water chemical variables and littoral macroivertebrate assemblages (Table 11, Fig. 6). Together these nine variables explained 24.2% of the variance not accounted for by geographic position, catchment landuse/type and other ecosystem variables (31.8% of the total variance was accounted for by these covariables). Three variables indicative of acid stress were included in the first nine variables selected. Water pH was selected in the first step and accounted for 6% of the variance in macroinvertebrate assemblages among lakes and the only other "acidification" indicator selected was alkalinity/acidity (3%, step 3). Although "landscape" effects were supposedly removed by running for example geographic position and catchment land use/type as covariables, a number of variables indicative of “lake types” were nonetheless selected in the constrained ordination. For example, minimum water temperature (2%), the nutrients PO_4-P (3%) and maximum NO_2+NO_3 (2%) and water color (2%) presumably indicate the influence of a latitudinal gradient on littoral macroinvertebrate assemblages in these “acid” reference lakes.

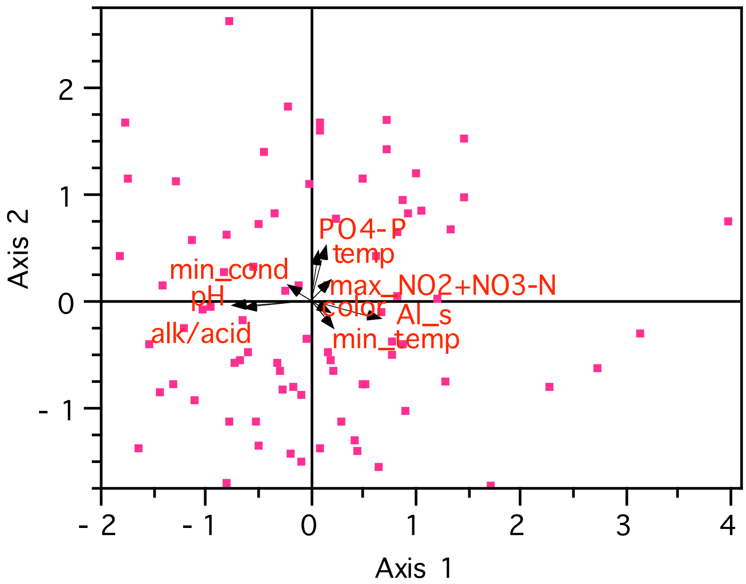

Figure 6. pCCA biplot of “acid” reference lakes and environmental variables, with all non water chemical variables run as covariables.

Table 11. Partial canonical correspondence analysis (pCCA) of littoral macroinvertebrates and environmental variables (mean and extreme (min or max) values. The influence of water chemical variables were analyzed by removing the effect of landscape and other descriptors by running these variables as covariables. All variables selected explained significant amounts of among-lake variability ($p < 0.001$)

Variable	λ1	model	step
pH	**0.06**	**0.06**	**1**
Alkalinity/Acidity	**0.05**	**0.03**	**3**
ANC	0.05		
ANCalk	0.05		
ANC/H+	0.05		
BC*/SO4*	0.04		
Ali	0.04		
Al3+	0.05		
H+Al3	0.05		
Ca/Ali	0.05		
SO4_IC	0.02		
Cl	0.03		
Al_s	**0.04**	**0.02**	**7**
TOCc	0.02		
Water temperature	**0.03**	**0.02**	**9**
Conductivity	0.03		

NO2+NO3-N	0.02		
Organic-N	0.02		
PO4-P	**0.03**	**0.03**	**2**
Total phosphorus	0.02		
Water color	**0.02**	**0.02**	**5**
Chlorophyll a	0.02		
min pH	0.05		
min Alkalinity/Acidity	0.04		
min ANC	0.05		
min ANCalk	0.05		
minANC/H+	0.05		
min BC*/SSA*	0.05		
min BC*/SO4*	0.05		
max Ali	0.04		
max Al3+	0.05		
max H+Al3	0.05		
min Ca/Ali	0.05		
max SO4_IC	0.02		
max Cl'	0.03		
max Al_s	0.04		
max TOC	0.02		
min Water temp	**0.02**	**0.02**	**6**
min Conductivity	**0.03**	**0.03**	**4**
max NO2+NO3-N	**0.02**	**0.02**	**8**
min Org-N	0.03		
min PO4-P	0.02		
min Tot-P	0.02		
max water color	0.02		
min Chlorophyll a	0.01		
total inertia		*1.446*	
Sum of all unconstrained eigenvalues (after fitting covariables)		*0.986*	
Sum of all canonical eigenvalues (after fitting covariables)		*0.239*	

Summary - The influence of variables indicative of "acid-stress" on littoral macroinvertebrate assemblages

The analyses above showed a number of variables indicative of acid-stress that might be used to describe and predict the variance in littoral macroivertebrate assemblages among lakes. Comparison of the four models that removed the effect of time (models 2-4) or other environmental variables other than water chemical values (model 5) resulted in a subset of five variables that explain significant amounts of the variance in macroinvertebrate assemblages.

In models 2 to 4, several years (1995 – 2002) were analyzed after partitioning the data set by ecoregion. Hence, these models should show the importance of variables that are not primarily affected by large-scale, ecoregion, effects such as climate. Surface water pH was found to be a significant predictor in two of the three regions; the exception being constrained ordination of taxa and lakes in the arctic/alpine ecoregion (Table 12). This finding was not too surprising given that the majority of S and N deposition occurs in the southwest parts of the country. Aside from pH, three variables also explained significant amounts of the among-lake variance. The ratio of base cations to sulfate concentration (BC/SO4) explained some of the residual variance not accounted for by pH in the boreal coniferous forest ecoregion (model 3), while both alkalinity/acidity and ANCalk were significant predictors in the mixed forest ecoregion (model 4). The results from model 5 show the importance of acid stress variables when all non-water chemical variables were removed. Two variables indicative of acid stress were found to be significant; namely, pH and buffering capacity (alkalinity/acidity).

Table 12. Selected chemical variables and their importance as predictors of littoral macroinvertebrate assemblages in "acid" reference lakes.

	Model 2	Model 3	Model 4	Model 5
pH		X	X	X
Alkalinity/Acidity			X	X
ANC				
ANCalk			X	
ANC/H+				
BC*/SO4*		X		
Ali				
Al3+				
H+Al3				
Ca/Ali				
SO4_IC				

<u>Establishing ecological breakpoints for selected biological metrics</u>
Lakes situated in the mixed forest ecoregion (ecoregion 3) were used to more closely evaluate the relationships between littoral macroinvertebrate assemblages in the "acid" reference lakes and selected chemical variables indicative of "acid" stress. Scores from the first axis of correspondence analysis of littoral macroinvertebrates, as well as two metrics commonly used to assess the ecological quality of lakes (Shannon diversity and Medin's acid index, Anonymous 1999) were regressed against mean annual lake pH, alkalinity/acidity, ANC and modeled inorganic Al (µg/L, WHAM-modeled, N. Cory pers. com.).

The three biological metrics showed similar response relationships to pH, alkalinity/acidity and ANC. Comparison of the CA scores, which reflect more of an unbiased relationship between community structure and acidity metrics, than either Shannon diversity (which depends on taxon richness and abundance) and Medin's acid index (which is a multimetric index that weights tolerant and sensitive taxa differently) were similar (Figs. 7 and 8). All three biological metrics showed a strong linear relationship with pH and fitting a quadratic function did not improve the fit (Table 13). Fitting a linear response curve gave r^2 values for CA scores, Shannon diversity and Medin's acid index of 0.74, 0.71 and 0.71, respectively (all p values were < 0.001), and root mean square error of the predictions (RMSE) were 0.525, 0.347 and 1.31 pH units, respectively, for the three metrics. Relationships between the biological metrics and alkalinity/acidity and ANC metrics were similar. Alkalinity/acidity and ANC explained 40% and 36% of the variance in CA axis 1 scores, 34% and 36% of the variance in Shannon diversity and 29% and 28% of the variance in Medin's acid index, respectively. Fitting a quadratic function improved the fit (r^2 values increase > 20%) and resulted in slightly lower errors.

The variance in the three metrics explained by inorganic Al (WHAM modeled) was similar to linear fits with ANC (i.e. r^2 values of 0.39, 0.43 and 0.39 for CA axis 1 scores, Shannon diversity and Medin's acid index, respectively). Fitting a quadratic function resulted in a slight improvement of model fitting (i.e. r^2 values of 0.46, 0.48 and 0.48 for CA axis 1 scores, Shannon diversity and Medin's acid index, respectively) and lower error.

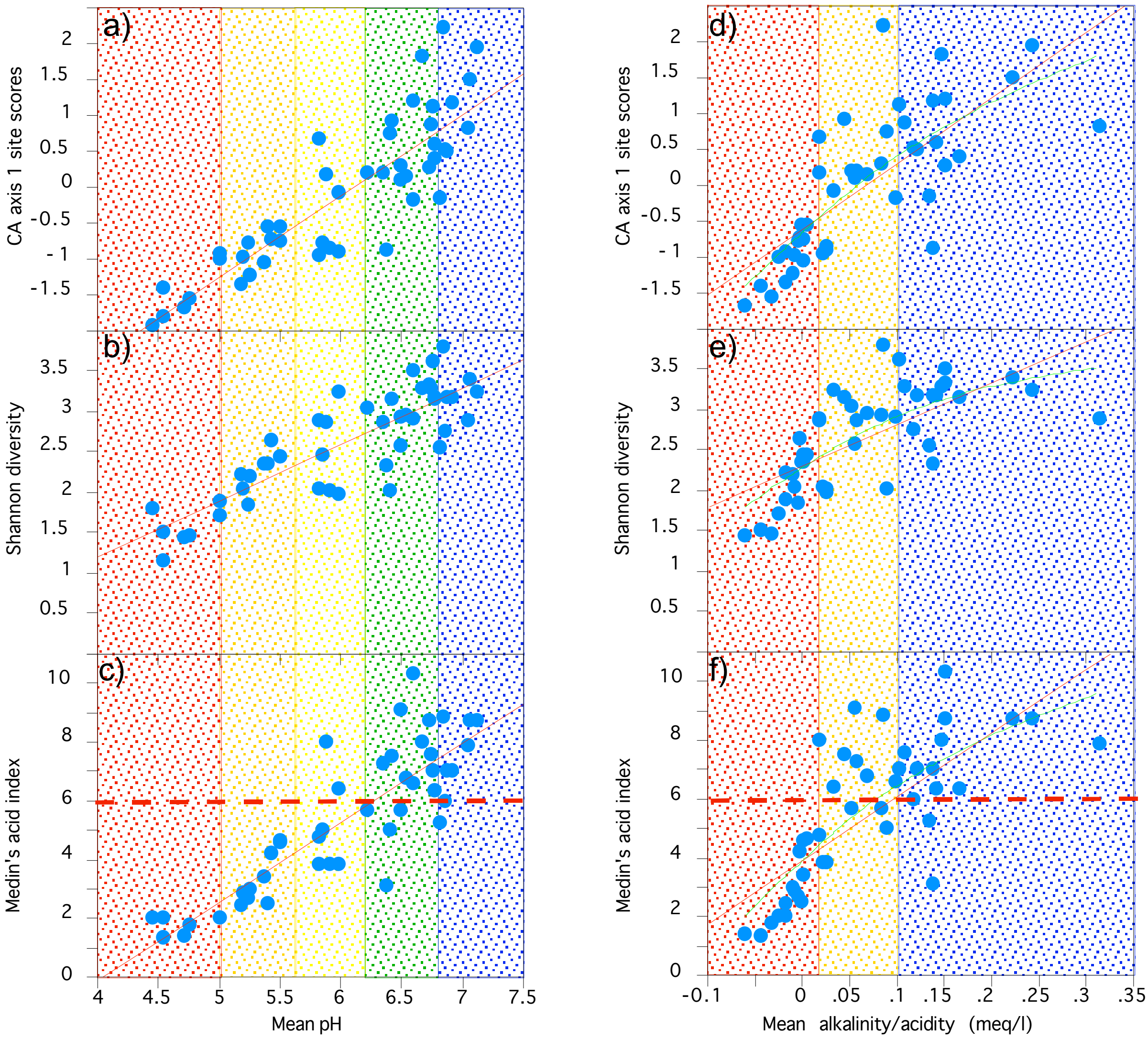

Figure 7. Regression plots of three biological metrics (CA scores, Shannon diversity and Medin's acid index) against pH and alkalinity/acidity for lakes situated in the mixed forest ecoregion. Curved lines show quadratic fit. The horizontal dashed line in figures c and f show the Medin's acid index value of 6.0; according to Ecological Criteria this designates the borderline where acid-stress effects may occur (Anonymous 1999).

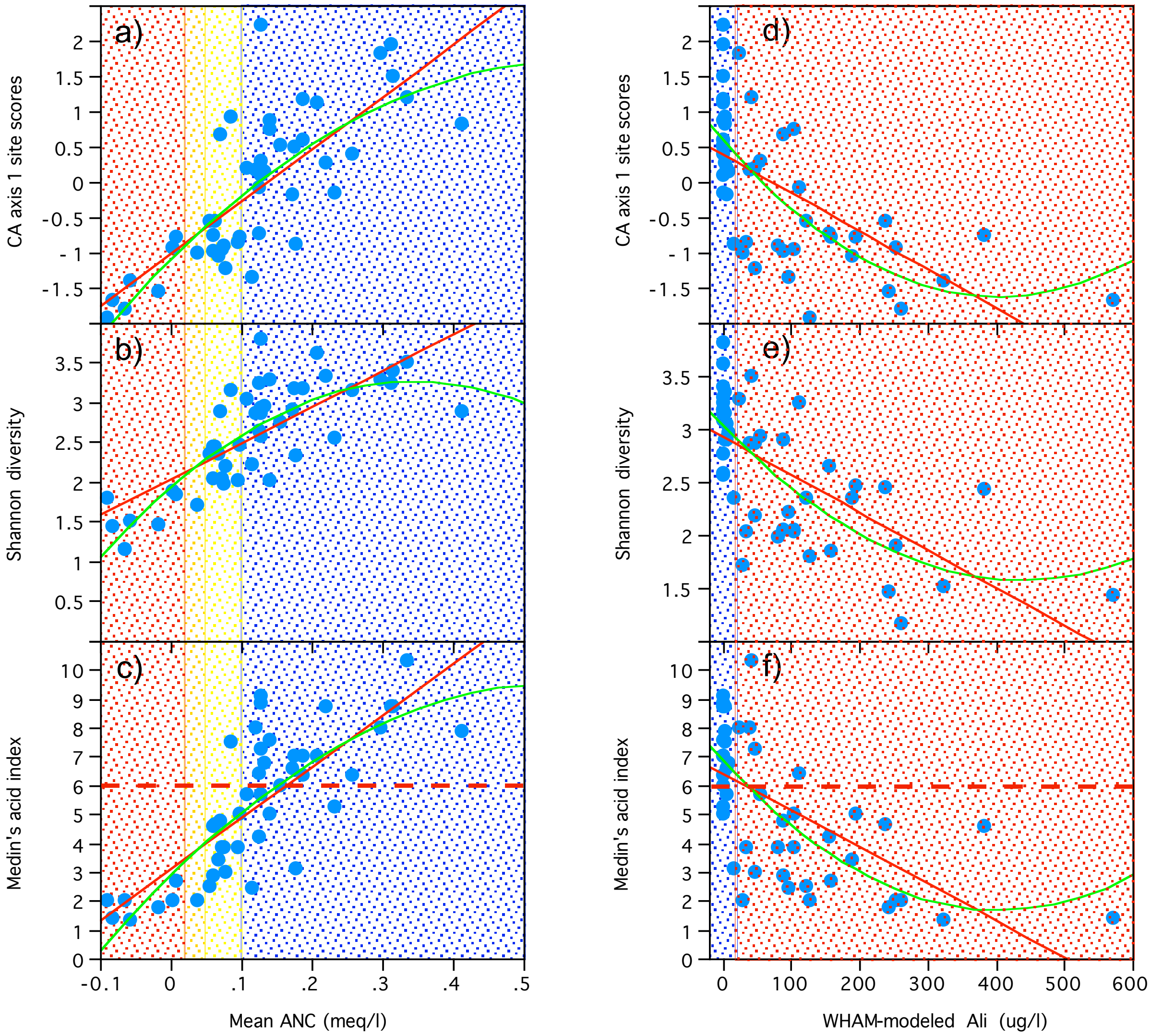

Figure 8. Regression plots of three biological metrics (CA scores, Shannon diversity and Medin's acid index) against ANC and inorganic Al for lakes situated in the mixed forest ecoregion. Curved lines show quadratic fit. The horizontal dashed line in figures c and f show the Medin's acid index value of 6.0; according to Ecological Criteria this designates the borderline where acid-stress effects may occur (Anonymous 1999).

Table 13. Summary statistics of CA scores (axis 1), Shannon diversity and Medin's acid index regressed against pH, alkalinity/acidity, ANC and WHAM-modeled inorganic Al (n = 48 lakes situated in the mixed forest ecoregion.

	pH	alkalinity/acidity	ANC	Inorganic Al
CA scores axis 1				
linear fit	p<0.001	p<0.001	p<0.001	p<0.001
R^2	0.74	0.4	0.36	0.39
RMSE	0.525	0.772	0.833	0.814
quadratic fit		p<0.001	p<0.001	p<0.001
R^2	0.74	0.63	0.59	0.46
RMSE	0.525	0.605	0.66	0.762
Shannon diversity				
linear fit	p<0.001	p<0.001	p<0.001	p<0.001
R^2	0.71	0.34	0.36	0.43
RMSE	0.347	0.501	0.52	0.489
quadratic fit		p<0.001	p<0.001	p<0.001
R^2	0.71	0.57	0.63	0.48
RMSE	0.346	0.404	0.39	0.464
Medin's index				
linear fit	p<0.001	p<0.001	p<0.001	p<0.001
R^2	0.71	0.29	0.28	0.39
RMSE	1.305	2.02	2.06	1.91
quadratic fit	p<0.001	p<0.001	p<0.001	p<0.001
R^2	0.71	0.62	0.62	0.46
RMSE	1.317	1.49	1.49	1.79

Setting class boundaries

All three biological metrics showed a funnel-shaped response when regressed against the three metrics of acidity use here (Figs. 7 and 8). Low variance, which can be interpreted as an indication of stress, was clearly evident at pH values < 5 and alkalinity/acidity and ANC values < 0.02 meq/L. In contrast to CA scores and Shannon diversity, Medin's acid index seemed to "level off" at pH < 5, which may be an artifact of the algorithm used (i.e. the lowest values possible). The finding that benthic macroinvertebrates in Swedish lakes are responding to acidity is not novel, but supports earlier studies of biological response to acidification. For example, Økland and Økland (1986) argued that gastropods were sensitive to changes in pH. These authors noted a loss of species richness between pH 6.15 and 5.9, and no gastropods were recorded at pH ≤ 5.2. Fish have also been used as an indicator for assessing the effects of acidification on aquatic ecosystems. Critical load work with fish has used an alkalinity of 0.05 meq/L as a threshold below which biological effects are predicted to occur.

Five classes were used to summarize the effects of pH on littoral macroinvertebrate communities (Table 14). Cutoffs for the five classes were pH < 5 (class 5 = extremely acid) and 5 < pH ≤ 5.6 (class

4 = very acid), 5.6 < pH ≤ 6.2 (class 3 = acid), 6.2 < pH ≤ 6.8 (class 2 = weakly acid) and pH > 6.8 (class 1 = neutral-alkaline). The "extremely acid" and "very acid" classes showed low variance around the regression line for all three biological metrics. Moreover, these two classes had low (< 5) or very low (< 2) values for Medin's acid index. Class 3 (acid) was indicated by an increase in residual variance around the regression line (all three metrics) and the upper boundary to this class constituted the intercept between the regression line and Medin's acid index value of 6.0. An index value of 6.0 is considered as the threshold level below which the probability of anthropogenic effects are expected (Anonymous 1999). The final two classes (weakly acid and neutral-alkaline) are defined at pH > 6.2. Medin's acid index showed marked variance in class 2, with values ranging from 3 to 10, while at pH values > 6.8 (class 1) only one lake had a acid score < 6.0 (Fig. 7c). A number of studies have shown biological effects at pH < 6 (e.g. Raddum and Fjellheim 1984; Henrikson and Medin 1986; Lindgdell and Engblom 1990; Lindgdell and Engblom 2002). The five classes suggested here agree to some extent with cut levels for pH currently used in Norway (i.e. 6.5, 6.0, 5.5 and 5.0; Lükewille 1997). A *caveat* in setting pH thresholds is that invertebrate sensitivity may be dependent on co-variables, such as Ca concentration, conductivity and color (Lonergan and Rasmussen 1996). Hence, caution should be exercised when interpreting "dose-response" relationships.

In contrast to pH, only three (alkalinity/acidity) or four (ANC) classes were established for the two metrics indicative of lake-water buffering capacity. Similar to the findings for pH, the residual variance was low at alkalinity/acidity and ANC values < 0.02 meq/L (class 5), and below this threshold seven lakes had acidity scores < 3. Class 3 (low alkalinity) had high variance around the regression line, with the majority of biological metrics placed above the regression line. The upper boundary of class 3 (i.e. 0.10 meq/L) was the intercept between the regression line and Medin's index value of 6. Class 2 (high alkalinity) had high variance around the regression line, and the majority of Medin's index values > 6. For ANC, an extra class (4 or very low alkalinity) was selected between 0.02 and 0.05 meq/L. Only one lake was situated in the ANC interval and above this cutoff Shannon diversity and Medin's index showed high variance. The finding that alkalinity/acidity and ANC values < 0.020 meq/L is critical for macroinvertebrates lends support to the study by Lien et al. (1995). These authors suggested 0.020 meq/L as the tolerance level for fish and macroinvertebrate assemblages of Norwegian surface waters. Raddum and Skjelkvåle (1995) in a review of European lakes and rivers argue, however, that this critical threshold is relevant only for systems with low Ca concentrations. For lakes and streams with high Ca concentrations (i.e. high ionic strength) the authors recommended a threshold of 0.050 meq/L.

Organism response to acidification can be complex, reflecting both the direct physiological effect of pH as well as the effects of associated metals, and indirect effects mediated through bottom-up processes (e.g. food availability). A number of studies have shown that macroinvertebrates, in particular mayflies, are affected directly by low pH and high concentrations of aluminium (e.g. Ormerod et al. 1987). Rosseland et al. (1990) found that aquatic organisms were affected by inorganic Al concentrations > 25 µg/L and these authors suggested this value as a lower threshold below which biological effects are negligible and a second concentration of 75 µg/L was suggested as an upper threshold where strong effects were predicted. Our findings showed that all three biological metrics were non-linearly related to inorganic Al concentration. Relatively high values were noted for all three biological metrics at inorganic Al concentrations < 20 µg/L. Hence, this was selected as a lower threshold, where Al-effects are expected to be low. At inorganic Al concentrations > 75 µg/L one lake had a Medin's acid index value > 6. Hence, although from our data we do not see a clear dose-response relationship, the second threshold of 75 µg/L as proposed by Rosselund et al. (1990) might also be recommended.

Table 14. Rational for setting class boundaries for pH, alkalinity/acidity, ANC and inorganic Al using lake (littoral) macroinvertebrate communities.

	pH	comments	**Alkalinity/Acidity (meq/L)**	comments	**ANC (meq/L)**	comments	**Inorganic Al (µg/L)**	comments
Class								
1	>6.8		n/a		n/a		n/a	
2	6.2-6.8	high residual variance; lower boundary at pH = 6.2, regression line intersects with Medin's index value of 6	> 0.10	high residual variance; lower boundary at alkalinity/acidity = 0.10 meq/L, regression line intersects with Medin's index value of 6	> 0.10	high residual variance; most Medin's index values > 6	n/a	
3	5.6-6.2	high residual variance; some Medin's index values > 6; upper boundary (pH = 6.2) is the intersection between regression line and Medin's index value of 6	0.02-0.10	high residual variance; several values > Medin index = 6; upper boundary (0.10 meq/L) is the intersection between regression line and Medin's index value of 6	0.05-0.10	high in residual variance; with the exception of one lakes, Medin's index values < 6	< 20 µg/L	below this thresh high CA axis 1 s Shannon diversit Medin's index va
4	5.0-5.6	low residual variance	n/a		0.02-0.05	only one lake in this interval	n/a	
5	< 5.0	Medin's index values < 2	< 0.02	low residual variance, Medin's index values < 5	< 0.02	low residual variance, Medin's index values < 3	> 20 µg/L	probability of anthropogenic ef increases (e.g. lo Medin's index va

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