Does freshwater macroinvertebrate diversity along a pH-gradient reflect adaptation to low pH?

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SUMMARY

1. The impacts of anthropogenic surface water acidification are much better known than those of natural acidity. Recent studies have indicated biodiversity is not degraded and species composition unaltered in naturally acidic compared to circumneutral watercourses.

2. Here, we use a geographically extensive dataset comprising sites in more than 200 Swedish streams to test whether the lack of effects on macroinvertebrate species diversity is due to exaptation and adaptation to natural acidity.

3. To this end, we modelled pH associated with spring flood episodes, which inflict the most challenging hydrochemical conditions to the biota. We compared taxonomic richness and species composition along the modelled pH gradient in northern Sweden, where acidity is largely natural, with southern Sweden, a region influenced by significant anthropogenic acidification.

4. We found Plecoptera richness did not respond to varying pH either in northern or southern Sweden. Ephemeroptera richness was sensitive to pH in both regions, while that of Trichoptera increased with increasing pH in southern Sweden, but decreased in the north. The taxonomic composition of Plecoptera changed along the pH gradient in both regions, whereas that of Ephemeroptera and Trichoptera changed more strongly with pH in southern Sweden.

5. Our results support the hypothesis that stream invertebrates are able to tolerate low pH through exaptation or adaptation, but that this capability varies among taxonomic groups.

Keywords: acidity, aquatic insects, community ecology, generalized linear mixed models, taxonomic richness

Introduction

Many studies have demonstrated negative effects of *anthropogenic acidification* of surface waters on biodiversity (e.g. Otto & Svensson, 1983; Guérold *et al.*, 2000; Braukmann, 2001), whereas the consequences of *natural acidity* have been comparatively little explored.

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Some studies suggest evolved widespread exaptation and possibly adaptation to low pH (Winterbourn & Collier, 1987; Collier *et al.*, 1990; Woodward, Jones & Hildrew, 2002; Dangles, Malmqvist & Laudon, 2004). An exaptation is a character used in a context unrelated to its origin, whereas adaptation to acidity implies exposure to acidic conditions over evolutionary time leading to the evolution of mechanisms that equip the biota for living in a putatively more stressful acidic environment. Hence, biodiversity in acidic freshwater systems is not necessarily impoverished where natural acidity has been widespread over extended periods (the evolutionary species pool

hypothesis, Pither & Aarssen, 2005). In fact, given that communities in naturally acidic freshwaters may be as diverse as those in circumneutral systems within the same geographical region, adaptation to low pH has been proposed (Collier *et al.*, 1990; Dangles *et al.*, 2004), but a more rigorous test is still wanting.

Natural acidity in freshwater environments is probably widespread. In Sweden, paleolimnological research suggests a long-term acidification trend over several thousand years (Renberg, Korsman & Anderson, 1993a; Korsman, 1999). Hydrochemical modelling implies natural pH declines during snow melt due to the dilution of alkalinity (Laudon & Bishop, 1999; Bishop, Laudon & Köhler, 2000) and pulsed release of organic acids (Laudon & Bishop, 2002a,b). These studies together imply acidity is mostly natural in large parts of northern Sweden with only a minor anthropogenic contribution to episodic acidity (Laudon & Bishop, 2002b; Laudon *et al.*, 2004). In contrast, recent anthropogenic acidification is widespread in streams in southern Sweden (Warfvinge & Bertills, 1999), but this area also shows limited natural background acidity, calcareous bedrock, eutrophication and historical increase in alkalinity due to land use change (Renberg & Hellberg, 1982; Renberg *et al.*, 1993a; Renberg, Korsman & Birks, 1993b). Hence, in southern Sweden the modern decline in pH likely provided less opportunity for marked evolutionary modifications of freshwater organisms in response to increasing acidity. Gene flow in some aquatic insects may be restricted even among close catchments (e.g. Hughes *et al.*, 1999). If gene flow between the macroinvertebrate populations in the two regions was limited in our study, then populations in the south and north are likely to have diverged genetically.

Taking macroinvertebrates as model organisms, we hypothesized that in naturally acidic streams they are adapted or exapted to tolerate low pH. We tested our hypothesis by a combination of modelling acidic episodes and analysing spatially large-scale datasets on benthic macroinvertebrate taxonomic richness, abundance, and assemblage structure, in a comparison of streams in southern and northern Sweden. If the biota were adapted, then the organisms found at acidic sites in northern Sweden would comprise an independent set of species evolved *in situ* in response to natural acidity compared to non-acidic sites in the north. We therefore predicted for adapted assemblages that the slope of the function relating

taxonomic richness to pH in northern Sweden should be less steep, zero, or even negative in contrast to southern Sweden. However, the slope should be weakly positive or near zero if macroinvertebrates were exapted, because in that case the traits evolved in a different context than to increase tolerance to an acidic environment. This means species found at acidic sites would make up a subset of pre-adapted species which evolved in non-acidic environments in response to environmental factors other than acidity. If, however, sensitivity to natural acidity was at hand, the slope should be positive and richness along the pH gradient not different between northern and southern Sweden. Species abundance distributions along a pH gradient in northern and southern Sweden should differ qualitatively indicating adaptation, exaptation, or sensitivity to naturally low pH in the same way as taxonomic richness.

Methods

We analysed data from two spatially large-scale surveys of 700 streams conducted across Sweden in 1995 and 2000 (Wilander *et al.*, 1998; Wilander, Johnson & Goedkoop, 2003). The detailed databases on water chemistry and macroinvertebrate species abundances constitute suitable material sufficient for testing our hypothesis. Each site was located at a different stream and sampled once in autumn. Similar riffles were sampled using standardized methods. At each site five replicate (later pooled) kick samples (500 μm mesh, sampling area 1 m^2, sampling time 1 min) were taken following Swedish standard procedures (Wilander *et al.*, 1998; Sandin, 2003; Wilander *et al.*, 2003). Benthic samples were preserved in the field in 70% ethanol, sorted in the laboratory, and subsampled when the sorting time exceeded 2 h. Macroinvertebrates were identified by professional taxonomists at six accredited and inter-calibrated laboratories (Wilander *et al.*, 1998; Sandin, 2003; Wilander *et al.*, 2003). All water chemistry analyses were performed by the Department of Environmental Assessment, Swedish University of Agricultural Sciences, Uppsala, using international or European standards (Wilander *et al.*, 1998, 2003).

The pH model

In Swedish streams, minimum pH is encountered during peak flow in spring (e.g. Laudon & Bishop,

1999; Laudon, Köhler & Bishop, 1999; Laudon & Bishop, 2002a). To account for the most stressful pH conditions at a site, we modelled water chemistry during spring flood episodes for two different scenarios: a wet and a dry year, since 1995 was a comparatively dry year, while 2000 was exceptionally wet (SMHI, 2000). For the two scenarios, we assumed a 25% and 50% decrease (Laudon *et al.*, 2004), respectively, in acid neutralizing capacity (ANC) of the stream water relative to baseflow conditions and a 50% and 100% increase, respectively, in total organic carbon (TOC) content (Laudon & Bishop, 2002b). As more than 95% of TOC consists of dissolved organic carbon (DOC) during both low and high flow (Köhler *et al.*, 2000), we substituted TOC for DOC to model the concentration of organic acid anions (Laudon, Westling & Bishop, 2000). Similar models have been used in other studies (Laudon & Bishop, 2002b; Laudon *et al.*, 2005). Finally, we assumed that inorganic monomeric aluminium species (Al^{n+}) did not significantly contribute to the buffering capacity of stream water (Köhler *et al.*, 2000) and therefore disregarded them in our computations. ANC can be calculated in two ways: (i) as the difference between base cations and strong acid anions, and (ii) as the difference between weak anions – where $RCOO^-$ are present as dissociated organic acid anions (Köhler *et al.*, 2000) – and weak cations (Laudon *et al.*, 2000).

$$\text{ANC} = [K^+] + [Na^+] + 2[Ca^{2+}] + 2[Mg^{2+}] \\ - 2[SO_4^{2-}] - [Cl^-] - [NO_3^-] \tag{1}$$

$$\text{ANC} = [HCO_3^-] + 2[CO_3^{2-}] + [RCOO^-] \\ + [OH^-] - [H^+] - n[Al^{n+}] \tag{2}$$

Combing these two equalities results in an expression which we rearranged to yield an equation (3) for the concentration of H^+ (Laudon *et al.*, 2000) – and thereby pH – as a function of ANC and DOC at a pCO_2 in equilibrium with the atmosphere.

$$[H^+] = [HCO_3^-] + 2[CO_3^{2-}] + [RCOO^-] + [OH^-] \\ - [K^+] - [Na^+] - 2[Ca^{2+}] - 2[Mg^{2+}] \\ + 2[SO_4^{2-}] + [Cl^-] + [NO_3^-] \tag{3}$$

pH was computed iteratively, as the concentrations of bicarbonate and organic carbon anions both depend on pH, and the results were used to model pH for the dry- and wet-year scenarios for 1995 and 2000 (Köhler

et al., 2000). The pH model predicts minimum spring flood pH successfully with a mean error <0.4 pH units (Buffam, Laudon & Bishop, 2005). Larger errors may occur where the TOC content is high, such as in headwater streams draining mires, but our study streams were of medium size and thus relatively groundwater influenced suggesting the errors remained mostly small.

We performed all analyses using only the model pH data from the dry-year scenario, because (i) they were highly correlated with those from the wet-year scenario and also with the raw data for both 1995 ($r_{dry/wet} = 0.95$, $t = 51.8$, d.f. = 281, $P < 0.01$ and $r_{dry/raw} = 0.78$, $t = 21.2$, d.f. = 281, $P < 0.01$) and 2000 ($r_{dry/wet} = 0.94$, $t = 47.4$, d.f. = 285, $P < 0.01$ and $r_{dry/raw} = 0.82$, $t = 24.2$, d.f. = 285, $P < 0.01$), (ii) pH during spring in dry years is generally lower (see above) and therefore supposedly biologically more challenging, (iii) because the model pH data from the dry-year scenario covered the larger range, and (iv) were based on assumptions relating more closely to the initial model development (Laudon & Bishop, 2002b; Laudon *et al.*, 2005). Mean annual pH data were not available and could therefore not be included in the analyses.

Site selection

We defined 'southern Sweden' to be the region in the south-west and 'northern Sweden' to comprise the northern half of the country reflecting differences in acid deposition (Fig. 1). In 1990, sulphate deposition in southern Sweden was 15–20 kg S ha^{-1} $year^{-1}$ compared with 3–5 kg S ha^{-1} $year^{-1}$ in the north (Warfvinge & Bertills, 1999). In 1997, deposition had decreased to 8–10 kg S ha^{-1} $year^{-1}$ and 2–5 kg S ha^{-1} $year^{-1}$ for southern and northern Sweden, respectively (Warfvinge & Bertills, 1999). We excluded streams in the interjacent part receiving intermediate levels of acid precipitation.

To avoid confounding, we also excluded all limed sites from the analyses, as liming buffers stream water and increases pH. We left out alkaline sites (>500 μeq ANC L^{-1}) – occurring largely in areas with calcareous bedrock in southern Sweden – and sites affected by intense agriculture (>25 μg tot P L^{-1}) prevailing in southern Sweden (Fölster, Sandin & Wallin, 2004). We set the cut-off level for total phosphorus to 35 μg L^{-1} when the TOC content exceeded 10 mg L^{-1}, as in such
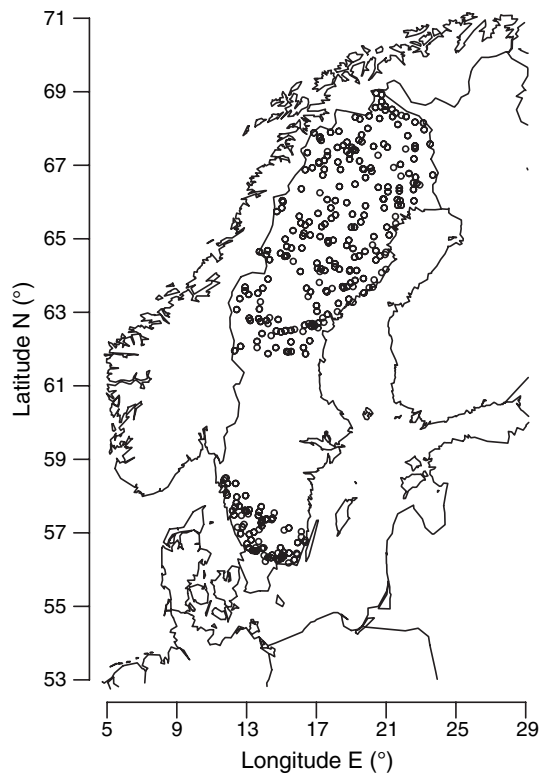
Fig. 1 Location of study sites. We defined 'southern Sweden' to be the region in the south-west and 'northern Sweden' to cover the northern half of Sweden.

sites elevated total phosphorus concentrations may be natural (Fölster *et al.*, 2004). We ended up with altogether 237 sites in 1995 and 242 sites in 2000. The pH-gradient of these sites ranged from 3.9 to 7.1 in the north and 4.1 to 6.7 in the south.

Data analysis

Because more species are recorded by chance alone when more individuals are sampled, we standardized taxonomic richness to a common number of individuals using rarefaction. We based the rarefaction on individuals because only pooled species-abundance data were available (Gotelli & Colwell, 2001). We aimed at including as many sites as possible and yet maximizing the reliability of detecting relevant differences in richness among different assemblages. Hence, we estimated richness at 200 individuals (Vinson & Hawkins, 1996), excluding sites tallying fewer individuals. In analyses of individual insect orders, we used observed taxonomic

richness, because the number of individuals was lower than 200.

We fitted generalized linear mixed models (GLMMs) to the data assuming a quasi-Poisson error distribution (Venables & Ripley, 2002) with 'rarefied taxonomic richness', 'observed taxonomic richness', and the square root of the 'abundance' for all taxonomic groups pooled and for stoneflies (Plecoptera), mayflies (Ephemeroptera), and caddisflies (Trichoptera) separately as dependent variables. The quasi-Poisson error distribution reflected the nature of the count data and allowed us to model the dispersion factor at the same time (Venables & Ripley, 2002). We fitted the two-level factor 'region' (southern and northern Sweden) as a fixed variable and 'model pH' as a covariate with year (1995 and 2000) as random factor. A principal component analysis of hydrochemical, topographical, geographical and structural environmental variables (substrate, aquatic and riparian vegetation) suggested that longitude – as proxy of other ecological factors – and the concentration of nitrate accounted for a substantial proportion of the environmental gradients. Consequently, we additively fitted 'longitude' and the 'concentration of nitrate' as independent covariates in the GLMMs to account for variation caused by these factors. The covariates were included additively into the GLMMs because environmental data were available for each site, but no replicate data were available for fitting interaction terms. Last, we implemented an autoregressive correlation structure for the GLMMs to account for potential spatial dependence among the study sites (Legendre, 1993; Pinheiro & Bates, 2000). We used penalized quasi-likelihood approximations for maximum-likelihood estimations (Venables & Ripley, 2002; Pinheiro *et al.*, 2005). The significance of the variables region, model pH, and their interaction in explaining variation in taxonomic richness and macroinvertebrate abundance was assessed using a conditional *F*-test, as *likelihood ratio* tests appear to be anti-conservative when assessing the significance of fixed effects in mixed models (Pinheiro & Bates, 2000). We used one-sided tests for our directional hypotheses. We assumed the treatment factor region to represent two aspects of acidity: natural in the north and with a substantial anthropogenic contribution in the south.

Similarly, we compared the abundances – square root transformed to meet model assumptions – of

those 33 genera and species between regions for which at least 100 individuals in no less than 10 streams were found in both southern and northern Sweden. For each taxon, we assessed whether the slope of the function relating abundance to model pH was smaller in northern compared to southern Sweden, which would indicate adaptation, and whether it was negative in northern Sweden, suggestive of exaptation. Running multiple tests increases the risk of obtaining significant results by chance alone, but this risk should not yield significance in more than three to four taxa at a level of 5% for type I errors (α).

We computed the number of mayfly, stonefly, and caddisfly taxa, other taxa, and all taxa taken together, across all northern and southern Swedish sites in both study years. For each category we also calculated how many taxa occurred in both regions, that is how many taxa were non-exclusive to either region. Because nearly four times as many sites were sampled in the north (175 in 1995, 203 in 2000) compared to the south (62 in 1995, 39 in 2000), we randomly subsampled the pool of northern sites to the size of the southern pool and used the observed taxonomic richness in northern Sweden to estimate the number of taxa common to both regions based on that subsample. We repeated this 1000 times.

We carried out canonical correspondence analyses (CCA) to compare species assemblages between regions and years. In order to relate these assemblages to acidity, we constrained the ordination to 'model pH' as the only explanatory variable. Consequently, variation along the first (canonical) axis was related to model pH, while variation along the second and all subsequent axes was related to other environmental gradients, that is we were able to assess how much variation was accounted for by model pH (Woodward *et al.*, 2002). To facilitate comparisons between northern and southern Sweden, we calculated the ratio between the eigenvalues of axes 2 and 1 (*axis ratio*), with values >1 indicating environmental gradients other than pH account for a larger proportion of the variation in species abundances than pH.

We ran CCA using Canoco for Windows 4.5 (ter Braak & Smilauer, 1997–2003) and computed rarefied taxonomic richness (Oksanen, Kindt & O'Hara, 2005) and fitted GLMMs (Pinheiro *et al.*, 2005) using the software package R 2.1.0 (R Development Core Team,

2005). All tests were performed at a probability level of 0.05 for type I errors.

Results

Overall 188 and 198 different taxa were identified across all study streams in southern Sweden in 1995 and 2000, respectively. More taxa were identified in northern Sweden (224 in 1995 and 270 in 2000). However, this was likely due to the higher number of sampled streams in the north: when comparing equal numbers of streams then more macroinvertebrate taxa were recorded in the south (Fig. 2). Fifty-two per cent (1995) and 54% (2000) of the taxa were common to both regions. Mayfly and stonefly taxa in northern Sweden outnumbered those in the south, but the number of caddisfly taxa was similar between the regions. On a per stream basis, observed total taxonomic richness and abundance did not differ between regions nor vary with pH (Table 1). Rarefied total taxonomic richness, however, was overall higher in the south (Table 1), where it tended to increase with increasing pH. Stonefly richness did not change with pH, but was higher in northern than in southern Sweden (Table 2, Fig. 3a). However, stonefly
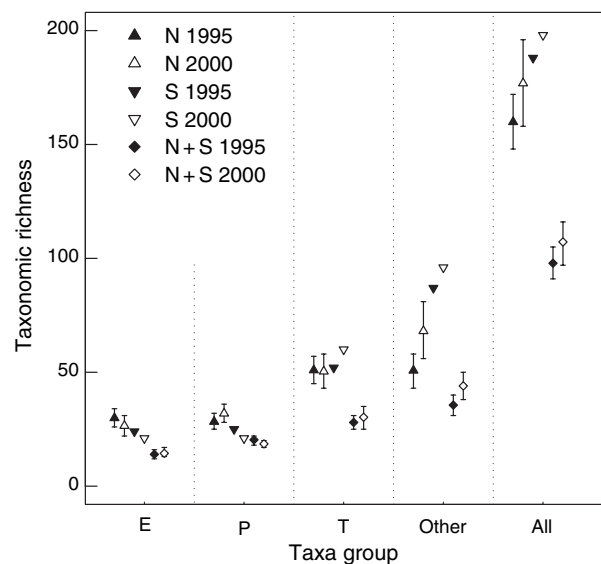

Fig. 2 Number of taxa observed in northern Sweden (N), southern Sweden (S), and number of taxa common to both regions (N + S) in 1995 and 2000. The error bars delimit the 2.5 and 97.5% quantiles of each group. Taxa groups are mayflies (E), stoneflies (P), caddisflies (T), other taxa (Other) and all taxa taken together (All).

Table 1 Conditional *F*-test statistics for region, pH, and their interaction in generalized linear mixed models for rarefied total taxonomic richness, observed total taxonomic richness and abundance square root transformed. One-sided tests were used for directional hypotheses

Effect	$F_{1,517}$	*P*-value
Rarefied total taxonomic richness		
Region	6.87	0.009
pH	0.41	0.525
Interaction	2.03	0.077
Observed total taxonomic richness		
Region	0.85	0.358
pH	0.71	0.400
Interaction	0.96	0.164
Abundance		
Region	1.39	0.239
pH	2.29	0.132
Interaction	1.55	0.107

abundance decreased with increasing pH and was also higher in the north (Fig. 3b). Mayfly richness and abundance both increased with increasing pH and

Table 2 Conditional *F*-test statistics for region, pH, and their interaction in generalized linear mixed models for stonefly, mayfly, and caddisfly taxonomic richness and the abundance of stoneflies, mayflies and caddisflies square root transformed. One-sided tests were used for directional hypotheses

Effect	$F_{1,517}$	*P*-value
Stonefly richness		
Region	57.58	<0.001
pH	1.41	0.236
Interaction	0.43	0.743
Mayfly richness		
Region	22.79	<0.001
pH	27.55	<0.001
Interaction	0.02	0.446
Caddisfly richness		
Region	1.29	0.256
pH	2.93	0.088
interaction	6.86	0.005
Stonefly abundance		
Region	16.90	<0.001
pH	14.95	<0.001
Interaction	2.75	0.049
Mayfly abundance		
Region	24.01	<0.001
pH	6.64	0.010
Interaction	1.55	0.893
Caddisfly abundance		
Region	0.63	0.429
pH	4.75	0.030
Interaction	10.62	0.001

were higher in northern Sweden (Table 2, Fig. 3c,d). Caddisfly richness and abundance showed diverging trends in the two regions (Table 2, Fig. 3e,f): while richness and abundance increased with increasing pH in southern Sweden, they decreased in the north. Contrary to stoneflies and mayflies, neither richness nor abundance of caddisflies was higher in the north (Fig. 2), although there was a trend towards overall higher richness and abundance with increasing pH (Table 2, Fig. 3e,f).

When genera and species were analysed separately, the slope relating abundance to pH was larger in southern than in northern Sweden in 12 (36%) out of the 33 analysed taxa (Table 3), suggesting adaptation. Exaptation was indicated by the negative slope in nine (27%) of the studied taxa in northern Sweden. Aside from stoneflies and caddisflies those taxa included mayflies, two beetle and two bivalve taxa.

In all ordinations, environmental factors other than model pH explained a higher proportion of the variation in species abundances than model pH alone (indicated by *axis ratio* >1, Fig. 4). However, model pH was clearly more important in southern than in northern Sweden in both years (59–67%) as the lower axis ratios suggested (Fig. 4). The species composition of mayflies and caddisflies was clearly more closely associated with environmental factors other than model pH in northern Sweden in both years (Fig. 4a,b,e,f). In stoneflies, however, species were mainly sorted along a model pH gradient in northern Sweden. In southern Sweden, mayfly and caddisfly species were proportionately more strongly associated with model pH (Fig. 4c,d,g,h), which, however, was not the case for stoneflies.

Discussion

Our results show distinctive patterns across regions and taxa. Disentangling the mechanisms behind these patterns is no easy task because they are not necessarily mutually exclusive. We have identified four mechanisms to which we devote the following discussion: (i) adaptation and exaptation to naturally low pH, (ii) exclusion of competitively inferior taxa from circumneutral systems, (iii) regional differences in macroinvertebrate faunas, and (iv) regional differences in the causes of acidity.

In our study stonefly richness, but not total taxonomic richness, conformed to the pattern expected for
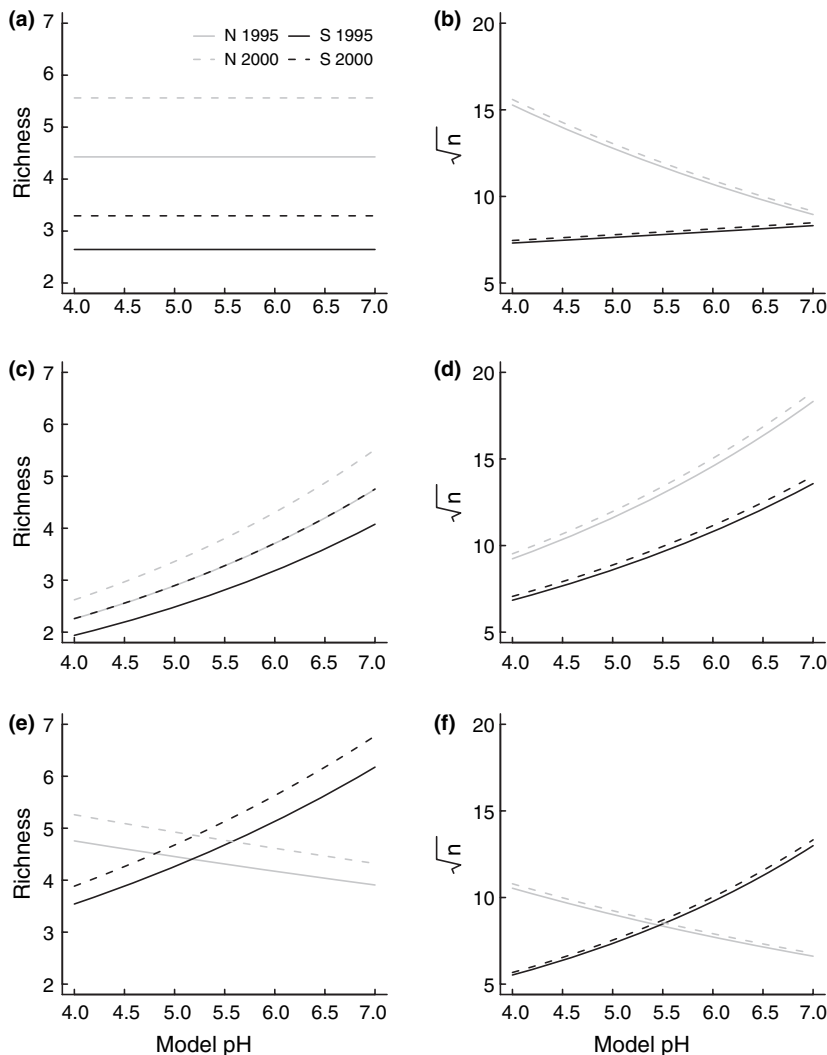
Fig. 3 Observed (a) stonefly, (c) mayfly and (e) caddisfly taxonomic richness and square root of the number of (b) stonefly, (d) mayfly and (f) caddisfly individuals versus model pH for streams in northern (N) and southern Sweden (S) in 1995 and 2000. The lines were drawn based on the model parameters and represent the model fits for the two regions in the 2 years. Note that the lines for northern Sweden in 1995 and southern Sweden in 2000 overlap in (c).

exaptation (Tables 1 & 2, Fig. 3a). The pattern indicating adaptation was apparent in caddisflies, where the slope of the function relating taxonomic richness to acidity was smaller in northern than in southern Sweden (Table 2, Fig. 3e). In mayflies, we found evidence for sensitivity (Table 2, Fig. 3c); though mayfly richness was overall higher in the north, possibly due to a larger regional species pool (Fig. 2). Generally, similar results have been previously reported from northern Sweden (Dangles *et al.*, 2004) and New Zealand, where also mayflies, unlike in our study, sustained high diversity at high acidity except for the sites with pH < 4.5 (Winterbourn & Collier, 1987; Collier *et al.*, 1990). Moreover, when particular genera and species were analysed separately, their abundance distributions in northern and southern

Sweden provided significant evidence for exaptation or adaptation in 19 (58%) of the studied taxa, including the allegedly more sensitive mayflies and bivalves (Table 3). The proportion of taxa showing significant evidence for exaptation or adaptation did not change if the analyses included only those taxa that were identified to the species level (57%). In general, our results are in agreement with other findings on species abundance distributions along an anthropogenic pH-gradient suggesting macroinvertebrate abundances generally increase with pH, except for stoneflies that show varying distributional patterns (Townsend, Hildrew & Francis, 1983; Tixier & Guérold, 2005).

Despite the fact that different sites represent considerable environmental variation, species

Table 3 Conditional *F*-test statistics (one-sided tests) for the interaction (i) between the region and pH and for the effect of pH in northern Sweden in generalized linear mixed models of the square root of the abundance of all genera and species tallying more than 100 individuals at ≥10 sites. One-sided tests were used for directional hypotheses

Taxon	$F_{i1,563}$	P_i	$F_{pH1,563}$	P_{pH}	Status
Bivalvia					
Sphaerium sp.	5.00	0.013	0.45	0.252	A
Pisidium sp.	3.99	0.023	0.20	0.329	A
Ephemeroptera					
Baetis rhodani	1.30	0.128	3.84	0.975	S
Baetis niger	4.98	0.013	0.04	0.420	A
Heptagenia sulphurea	0.61	0.218	0.71	0.201	S
Heptagenia fuscogrisea	1.05	0.153	1.89	0.085	(E)
Leptophlebia sp.	3.04	0.041	0.52	0.236	A
Leptophlebia marginata	0.11	0.628	4.22	0.020	E
Leptophlebia vespertina	0.09	0.384	3.72	0.027	E
Plecoptera					
Taeniopteryx nebulosa	<0.01	0.513	6.94	0.004	E
Brachyptera risi	1.85	0.913	1.61	0.898	S
Protonemura meyeri	2.31	0.064	11.18	<0.001	(A), E
Amphinemura sulcicollis	0.06	0.402	3.18	0.038	E
Amphinemura borealis	0.34	0.281	0.10	0.377	S
Nemoura sp.	0.64	0.788	2.60	0.054	(E)
Nemoura cinerea	0.89	0.173	0.33	0.284	S
Nemoura avicularis	4.70	0.015	0.03	0.429	A
Leuctra hippopus	2.82	0.047	10.84	0.001	A, E
Leuctra fusca/digitata/ hippopus	5.82	0.008	1.36	0.122	A
Isoperla sp.	0.90	0.828	1.22	0.865	S
Isoperla difformis	0.09	0.385	1.71	0.096	(E)
Coleoptera					
Hydraena sp.	6.64	0.005	2.69	0.949	A
Elmis aenea	0.14	0.352	5.44	0.990	S
Limnius volckmari	4.53	0.017	0.63	0.215	A
Oulimnius troglodytes/ tuberculatus	0.23	0.316	1.21	0.864	S
Trichoptera					
Rhyacophila nubila	0.90	0.828	<0.01	0.498	S
Plectrocnemia sp.	1.50	0.110	6.47	0.006	E
Polycentropus flavomaculatus	0.88	0.174	6.09	0.007	E
Hydropsyche pellucidula	4.99	0.013	1.28	0.130	A
Hydropsyche siltalai	6.46	0.006	3.18	0.037	A, E
Potamophylax sp.	0.09	0.620	6.30	0.994	S
Lepidostoma hirtum	2.35	0.063	0.04	0.421	(A)
Sericostoma personatum	3.57	0.030	0.11	0.628	A

Inferred status: A, adapted; E, exapted; S, sensitive.
Status in brackets indicates marginal significance
(0.05 ≤ P < 0.10).

composition apparently did respond to the pH gradient. In the ordination analyses (Fig. 4) the pH gradient explained a greater proportion of the variation in mayfly and caddisfly species abundances in southern than in northern Sweden, indicating, in line with the

taxonomic richness data, a higher degree of sensitivity to low pH in the south. This geographic differentiation suggests these insects have not evolved responses to acidity to the same extent in the south as in the north, but are represented according to their intrinsic tolerance levels. Acidity can thus be a major structuring force for mayfly and caddisfly assemblages in recently acidified streams. In stoneflies, however, pH strongly determined species composition. The presence of different species at acidic and circumneutral conditions suggests either adaptation or exaptation, as stonefly richness was not impaired by low pH in either region (Table 2, Fig. 3a) and a number of adapted and exapted taxa were identified (Table 3). Environmentally tolerant species may be competitively excluded from more benign conditions across a wide range of taxa (Bovbjerg, 1970; Liancourt, Callaway & Michalet, 2005). Therefore, tolerant macroinvertebrates might be suppressed at circumneutral environments potentially resulting in the development of either acid-sensitive assemblages or assemblages that are able to persist at acidic sites.

Our study also clearly demonstrates how the potential for adaptation varies among taxonomic groups. Whereas mayflies generally showed sensitivity to acidity and stoneflies exaptation or adaptation, the patterns in caddisfly distribution along pH gradients implied adaptation in northern Sweden. These disparate responses suggest phylogenetic constraints in the adaptability to acidity in mayflies. Complete lack of adaptability to low pH is, however, unlikely in mayflies given the presence of relatively acid tolerant and evidently exapted or adapted species, such as *Leptophlebia marginata* (L.) and *Baetis niger* (L.) (Table 3), and the high diversity observed at acidic sites in New Zealand (Winterbourn & Collier, 1987). Considering the different glacial histories of Fennoscandia – which was periodically fully covered by ice – and New Zealand – which harboured ice-free habitat over several glaciations (c.f. McGlone, 1985) – suggests that not all mayfly taxa have been equally capable of evolving in response to acidity or that some have evolved comparatively slowly.

The composition of the macroinvertebrate faunas in the two regions is partly different probably as a result of different biogeographical histories. Colonization of the region took place via two major routes: from the northeast and from the south (e.g. Lillehammer, 1985),
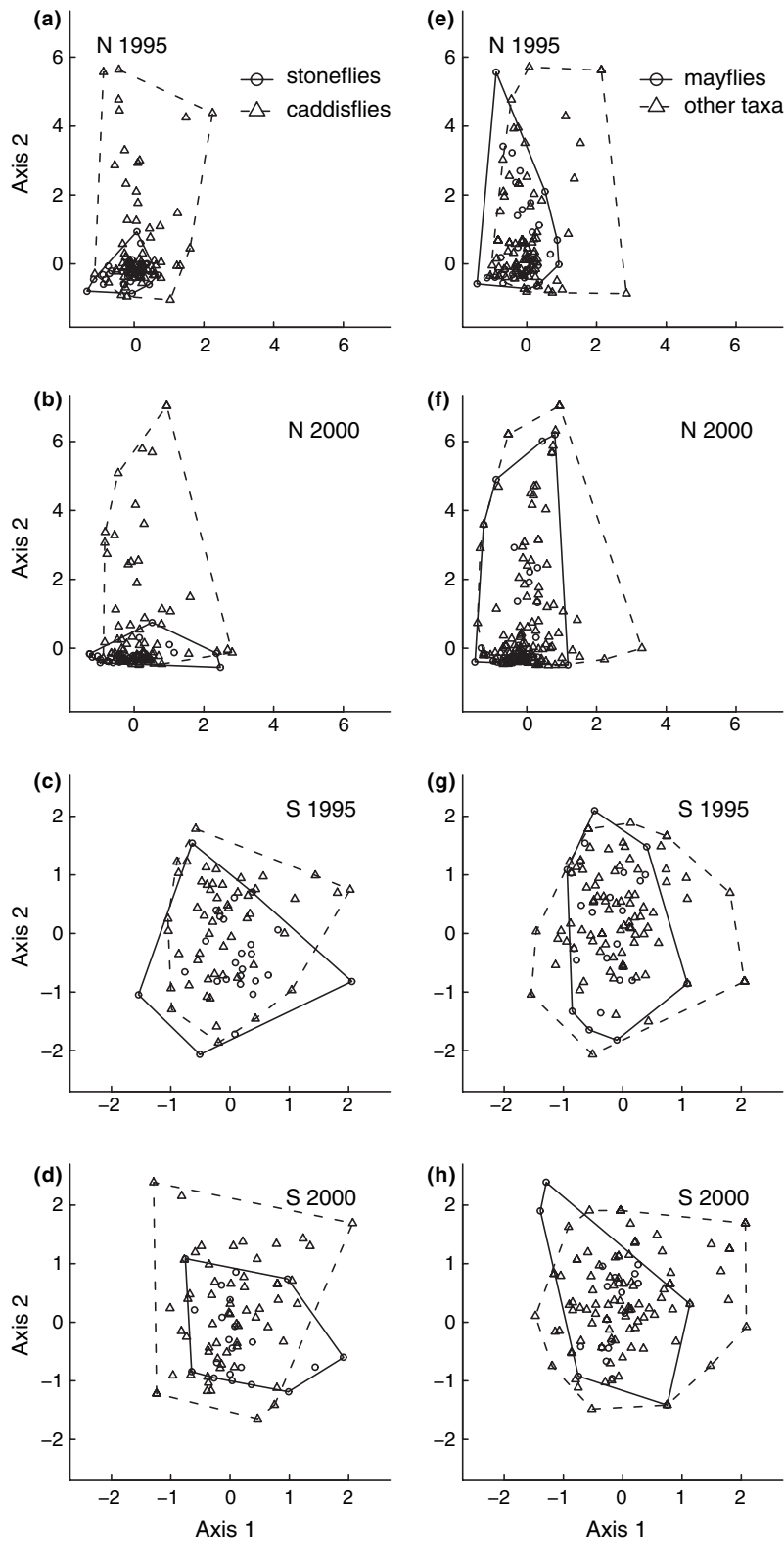
Fig. 4 Species scores from CCA on community data from northern (N) and southern Sweden (S) in 1995 and 2000. Model pH decreases along the first axis towards the right. The symbols for the different taxa, stoneflies and caddisflies (a–d) and mayflies and other taxa (e–h), are enclosed in separate convex-polygons to illustrate their distribution in the ordination space. (a, e) Eigenvalues: Axis 1 = 0.071, Axis 2 = 0.368, *axis ratio* = 5.2, $p_{(axis1)}$ = 0.002. (b, f) Eigenvalues: Axis 1 = 0.099, Axis 2 = 0.503, *axis ratio* = 5.1, $p_{(axis1)}$ = 0.002. (c, g) Eigenvalues: Axis 1 = 0.119, Axis 2 = 0.421, *axis ratio* = 3.5, $p_{(axis1)}$ = 0.156. (d, h) Eigenvalues: Axis 1 = 0.136, Axis 2 = 0.410, *axis ratio* = 3.0, $p_{(axis1)}$ = 0.062.

which has contributed to the extant distribution potentially explaining the response observed in caddisfly richness (Fig. 3e). However, geographically distinct faunas do not explain why mayflies and stoneflies differed in richness between the regions but nevertheless showed similar distributional patterns with respect to pH (Fig. 3a,c). Moreover, we found evidence for exaptation or adaptation in more than half of the taxa analysed individually (Table 3). Thus, irrespective of where and when the species traits were acquired, our findings more likely reflect differences in physiological tolerances through adaptation or exaptation than the legacy of biogeographic processes.

Our pH model does not account for the deposition of mineral acids which bias the pH estimates conservatively for southern Sweden where deposition is higher due to the proximity to industrial sources. However, as we base our judgment primarily on qualitative trends, we do not think this has any strong effects on our conclusions. Slow chemical (Stoddard *et al.*, 1999; Moldan *et al.*, 2001; Skjelkvåle *et al.*, 2001) and modest biological recovery in southern Sweden may further bias our results, but again in a conservative direction.

The underlying causes of acidity differ between southern and northern Sweden, as acidity in the south is brought about by both anthropogenic and natural factors (Renberg & Hellberg, 1982; Renberg *et al.*, 1993a,b), while it is largely natural in the north (Renberg *et al.*, 1993a; Korsman, 1999; Laudon & Bishop, 2002b; Laudon *et al.*, 2004). Mineral acids and labile aluminium could therefore be more prevalent in southern Sweden. Thus, the contribution of DOC to acidity, and thereby also its ameliorating effects (Kullberg, 1992; Kullberg *et al.*, 1993), would be less common in the south. Consequently, pH-related toxicity here might be higher explaining, for instance, why caddisfly richness and abundance showed diverging trends between the two regions (Fig. 3e,f). However, mayfly and stonefly richness and mayfly abundance did not show such diverging trends (Fig. 3a–d) suggesting the causes of low pH are not generally significant. Likewise, the chemical causes of acidity did not appear to influence the macroinvertebrate species distributions in streams in New Zealand (Winterbourn & McDiffett, 1996).

In natural experiments as in our study various factors including differences in the biogeographical colonization history and sources of acidity may often confound the treatment. We believe our study nevertheless constitutes an important contribution through testing the hypothesis in a relevant natural setting including a wide variety of environmental and ecological interactions rather than under controlled experimental conditions with potentially little ecological realism.

Three criteria need to be met to demonstrate adaptation to natural acidity: (i) it is a consequence of natural selection, (ii) it results in enhanced fitness, and (iii) it is functional. To establish exaptation to natural acidity, however, only (ii) and (iii) need to be demonstrated, because exaptation arises as a by-product of natural selection. Whether a trait evolved through natural selection for a particular biological function can, however, only be tested in the laboratory. Hence, our conclusions relate primarily to fitness and functionality. In summary, our study provides different lines of evidence concordant with the species pool hypothesis (Pither & Aarssen, 2005) including species richness distributions in caddisflies, region-specific abundance distributions in particular macroinvertebrate taxa and differences in species assemblages. Although none of these verifications alone demonstrates adaptation unequivocally, their combination supports our conclusion of evolved traits allowing macroinvertebrates to form diverse assemblages in naturally acidic streams in northern Sweden.

References

Bishop K.H., Laudon H. & Köhler S. (2000) Separating the natural and anthropogenic components of spring flood pH decline: a method for areas that are not chronically acidified. *Water Resources Research*, **36**, 1873–1884.

Bovbjerg R.V. (1970) Ecological isolation and competitive exclusion in two crayfish (*Orconectes virilis* and *Orconectes immunis*). *Ecology*, **51**, 225–236.

ter Braak C.J.F. & Smilauer P. (1997–2003) *Canoco for Windows, Biometrics*. Plant Research International, Wageningen, The Netherlands.

Braukmann U. (2001) Stream acidification in South Germany – chemical and biological assessment methods and trends. *Aquatic Ecology*, **35**, 207–232.

Buffam I., Laudon H. & Bishop K. (2005) *Development of an Empirical Model to Predict pH in Streams During Spring Flood in Northern Sweden*. Department of Environmental Assessment, Swedish University of Agricultural Sciences, Environmental Quality Criteria Report-Rivers 2005: 4, Uppsala, Sweden.

Collier K.J., Ball O.J., Graesser A.K., Main M.R. & Winterbourn M.J. (1990) Do organic and anthropogenic acidity have similar effects on aquatic fauna? *Oikos*, **59**, 33–38.

Dangles O., Malmqvist B. & Laudon H. (2004) Naturally acid freshwater ecosystems are diverse and functional: evidence from boreal streams. *Oikos*, **104**, 149–155.

Fölster J., Sandin L. & Wallin M. (2004) *A Suggestion to a Typology for Swedish Inland Surface Waters According to the EU Water Framework Directive*. Department of Environmental Assessment, Swedish University of Agricultural Sciences, report 13, Uppsala, Sweden.

Gotelli N.J. & Colwell R.K. (2001) Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters*, **4**, 379–391.

Guérold F., Boudot J.-P., Jacquemin G., Vein D., Merlet D. & Rouiller J. (2000) Macroinvertebrate community loss as a result of headwater stream acidification in the Vosges Mountains (N-E France). *Biodiversity and Conservation*, **9**, 767–783.

Hughes J.M., Mather P.B., Sheldon A.L. & Allendorf F.W. (1999) Genetic structure of the stonefly, *Yoraperla brevis*, populations: the extent of gene flow among adjacent montane streams. *Freshwater Biology*, **41**, 63–72.

Köhler S., Laudon H., Wilander A. & Bishop K. (2000) Estimating organic acid dissociation in natural surface waters using total alkalinity and TOC. *Water Research*, **34**, 1425–1434.

Korsman T. (1999) Temporal and spatial trends of lake acidity in northern Sweden. *Journal of Paleolimnology*, **22**, 1–15.

Kullberg A. (1992) Benthic macroinvertebrate community structure in 20 streams of varying pH and humic content. *Environmental Pollution*, **78**, 103–106.

Kullberg A., Bishop K.H., Hargeby A., Jansson M. & Petersen R.C. (1993) The ecological significance of dissolved organic carbon in acidified waters. *Ambio*, **22**, 331–337.

Laudon H. & Bishop K.H. (1999) Quantifying sources of acid neutralisation capacity depression during spring flood episodes in Northern Sweden. *Environmental Pollution*, **105**, 427–435.

Laudon H. & Bishop K.H. (2002a) Episodic stream water pH decline during autumn storms following a summer drought in northern Sweden. *Hydrological Processes*, **16**, 1725–1733.

Laudon H. & Bishop K.H. (2002b) The rapid and extensive recovery from episodic acidification in northern Sweden due to declines in SO_4^{2-} deposition. *Geophysical Research Letters*, **29**, no. 1594.

Laudon H., Köhler S. & Bishop K.H. (1999) Natural acidity or anthropogenic acidification in the spring flood of northern Sweden? *The Science of the Total Environment*, **234**, 63–73.

Laudon H., Westling O. & Bishop K. (2000) Cause of pH decline in stream water during spring melt runoff in northern Sweden. *Canadian Journal of Fisheries and Aquatic Sciences*, **57**, 1888–1900.

Laudon H., Westling O., Bergquist A. & Bishop K. (2004) Episodic acidification in northern Sweden: a regional assessment of the anthropogenic component. *Journal of Hydrology*, **297**, 162–173.

Laudon H., Hruška J., Köhler S. & Krám P. (2005) Retrospective analyses and future predictions of snowmelt-induced acidification: example from a heavily impacted stream in the Czech Republic. *Environmental Science & Technology*, **39**, 3197–3202.

Legendre P. (1993) Spatial autocorrelation: trouble or new paradigm? *Ecology*, **74**, 1659–1673.

Liancourt P., Callaway R.M. & Michalet R. (2005) Stress tolerance and competitive-response ability determine the outcome of biotic interactions. *Ecology*, **86**, 1611–1618.

Lillehammer A. (1985) Zoogeographical studies on Fennoscandian stoneflies (Plecoptera). *Journal of Biogeography*, **12**, 209–221.

McGlone M.S. (1985) Plant biogeography and the late Cenozoic history of New Zealand. *New Zealand Journal of Botany*, **23**, 723–749.

Moldan F., Wright R.F., Löfgren S., Forsius M., Ruoho-Airola T. & Skjelkvåle B.L. (2001) Long-term changes in acidification and recovery at nine calibrated catchments in Norway, Sweden and Finland. *Hydrology and Earth System Sciences*, **5**, 339–349.

Oksanen J., Kindt R. & O'Hara R.B. (2005) *Vegan: Community Ecology Package version 1.6–9*, http://cran.r-project.org/ Last accessed on 4 July 2007.

Otto C. & Svensson B.S. (1983) Properties of acid brown water streams in South Sweden. *Archiv für Hydrobiologie*, **99**, 15–36.

Pinheiro J.C. & Bates D.M. (2000) *Mixed-Effects Models in S and S-PLUS*. Springer-Verlag, New York.

Pinheiro J., Bates D., Debroy S. & Sarkar D. (2005) *nlme: Linear and Nonlinear Mixed Effects Models*, R package version 3.1–57. http://cran.r-project.org/. Last accessed on 4 July 2007.

Pither J. & Aarssen L.W. (2005) The evolutionary species pool hypothesis and patterns of freshwater diatom diversity along a pH gradient. *Journal of Biogeography*, **32**, 503–513.

R Development Core Team (2005) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Renberg I. & Hellberg T. (1982) The pH history of lakes in southwestern Sweden, as calculated from the subfossil diatom flora of the sediments. *Ambio*, **11**, 30–33.

Renberg I., Korsman T. & Anderson N.J. (1993a) A temporal perspective of lake acidification in Sweden. *Ambio*, **22**, 264–271.

Renberg I., Korsman T. & Birks H.J.B. (1993b) Prehistoric increases in the pH of acid-sensitive Swedish lakes caused by land-use changes. *Nature*, **362**, 824–827.

Sandin L. (2003) Benthic macroinvertebrates in Swedish streams: community structure, taxon richness, and environmental relations. *Ecography*, **26**, 269–282.

Skjelkvåle B.L., Mannio J., Wilander A. & Andersen T. (2001) Recovery from acidification of lakes in Finland, Norway and Sweden 1990–1999. *Hydrology and Earth System Sciences*, **5**, 327–337.

SMHI (2000) Regn, regn och åter regn. *Väder och Vatten*, **13**, 2–14.

Stoddard J.L., Jeffries D.S., Lükewille A. *et al.* (1999) Regional trends in aquatic recovery from acidification in North America and Europe. *Nature*, **401**, 575–578.

Tixier G. & Guérold F. (2005) Plecoptera response to acidification in several headwater streams in the Vosges Mountains (northeastern France). *Biodiversity and Conservation*, **14**, 1525–1539.

Townsend C.R., Hildrew A.G. & Francis J. (1983) Community structure in some southern English streams: the influence of physicochemical factors. *Freshwater Biology*, **13**, 521–544.

Venables W.N. & Ripley B.D. (2002) *Modern Applied Statistics with S*. Springer-Verlag, New York.

Vinson M.R. & Hawkins C.P. (1996) Effects of sampling area and subsampling procedure on comparisons of taxa richness among streams. *Journal of the North American Benthological Society*, **15**, 392–399.

Warfvinge P. & Bertills U. (1999) *Recovery from acidification in the natural environment: present knowledge and future scenarios*. Swedish Environmental Protection Agency, report 5034, Stockholm.

Wilander A., Johnson R.K., Goedkoop W. & Lundin L. (1998) *Riksinventering 1995-En synoptisk studie av vattenkemi och bottenfauna i svenska sjöar och vattendrag*. Swedish Environmental Protection Agency, report 4813, Uppsala.

Wilander A., Johnson R.K. & Goedkoop W. (2003) *Riksinventering 2000-En synoptisk studie av vattenkemi och bottenfauna i svenska sjöar och vattendrag*. Department of Environmental Assessment, University of Agricultural Sciences, report 2003:1, Uppsala.

Winterbourn M.J. & Collier K.J. (1987) Distribution of benthic invertebrates in acid, brown water streams in the South Island of New Zealand. *Hydrobiologia*, **153**, 277–286.

Winterbourn M.J. & McDiffett W.F. (1996) Benthic faunas of streams of low pH but contrasting water chemistry in New Zealand. *Hydrobiologia*, **341**, 101–111.

Woodward G., Jones J.I. & Hildrew A.G. (2002) Community persistence in Broadstone Stream (U.K.) over three decades. *Freshwater Biology*, **47**, 1419–1435.