Classification of boreal stream riffle macroinvertebrate assemblages and acid stress

By

Richard K. Johnson

Department of Environmental Assessment
Swedish University of Agricultural Sciences
Box 7050   SE 750 07 Uppsala

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INTRODUCTION

For several decades, emissions of N and S have deleteriously affected the ecological integrity of surface waters in Sweden and elsewhere. In the early 1990s it was estimated, for example, that some 14,000 or 15% of Swedish lakes with a surface area < 1 km^2 and about one-fifth of all watercourses could be regarded as being adversely affected by acidification (Bernes 1991). Although natural recovery of water chemistry has been documented in a number of lake ecosystems in Sweden (Wilander 1997) and across Europe (Stoddard et al. 1999), and more recently changes in lake biology have also been reported (e.g. Raddum et al. 2001), acidification is still considered as a major pressure deleteriously affecting the structure and function of lakes and streams in Sweden.

To better understand how to manage, protect and restore the structure, function and biodiversity of aquatic habitats, more knowledge is needed on how organisms respond to human-induced as well as natural environmental changes. In a recent study, Johnson et al. (2004) evaluated the response of lake littoral macroinvertebrate communities to natural and human-induced (acidification) stressors. These authors found that lake macroinvertebrate communities were responding to large-scale (regional patterns in landscape type/use) as well as more site-specific patterns in water chemistry. In particular, Johnson et al. (2004) showed that the littoral communities were best correlated with pH and buffering capacity (alkalinity/acidity). This study was designed to correlatively assess relationships between stream-riffle macroinvertebrate communities and physico-chemical metrics (variables) indicative of acid stress. Moreover, as a follow-up study of that done by Johnson et al. (2004), focus here is on determining if riffle macroinvertebrate communities are responding in a similar way to acidification stress and, if so, to determine if the ecological classification or threshold levels suggested by Johnson et al. (2004) are applicable for stream (riffle) macroinvertebrate communities.

METHODS

Study streams
The streams included in this study are taken from the national stream survey done in 1995 and 2000 and "reference" streams that are monitored on an annual basis. For more information regarding the study streams please refer to the Department of Environmental Assessment's website (**www.ma.slu.se**).

Data from national surveys - Macroinvertebrate communities of 694 and 706 streams were sampled in 1995 and 2000, respectively, as part of the Swedish national stream survey (Wilander *et al.*, 1998; Henriksen *et al.*, 1998; Johnson & Goedkoop, 2000; Wilander *et al.*, 2003). A number of factors suggested that this dataset was sufficiently robust for examining relationships between macroinvertebrate assemblages and water chemistry. Streams were selected randomly; thus, the samples should be representative of the population of streams sampled. However, in selecting stream sites two size classes were used (catchment area classes of 15 to 50 and 50 to 250 km^2), hence streams below or above these size classes are not part of the universe of this study. A number of measures were also taken to reduce natural or operator-induced variability. To reduce within-site variability, sample collection was restricted both temporally (autumn) and spatially (stony habitats), and samples were collected using standardised kick-sampling (European Committee for Standardisation, 1994) with a handnet (0.5 mm mesh size). A more detailed description of stream selection as well as field and laboratory procedures is given in Wilander *et al.* (1998, 2003).

A composite sample consisting of five kick-samples (60 sec x 1 m for streams) was taken from each site (one site per stream) and pooled. The size of the area sampled varied between streams. Although the upstream/downstream length was the same (i.e. 10 m), the width of the area sampled equalled 50% of the stream width (i.e. stream edges were not sampled). Rarefraction-adjusted taxon richness (calculated using the computer program EcoSim ver. 7.0, Gotelli & Entsminger, 2001) and measured taxon richness were strongly correlated (Johnson et al. 2004), thus we consider the sampling to be comparable. All samples were sorted and the animals identified according to quality control and assurance protocols (Wilander *et al.*, 1998; 2003). Identification was done to the lowest taxonomic unit possible, usually to species or species groups, with the exception of oligochaetes and chironomids.

In addition to macroinvertebrate samples, water samples were collected and analyzed for a number of water chemistry variables (e.g. nutrients, water color, conductivity, base cations and anions and acidity metrics), following international (ISO) or European (EN) standards when available (Wilander *et al.*, 1998; 2003). The sites where macroinvertebrate samples were taken were also classified according to substratum particle size and vegetation, and the riparian zones (shoreline stretches, 50 m long and 5 m wide, adjacent to the areas sampled) and catchments were classified according land use and vegetation cover.

Data from "reference" sites - In the late 1990s, Sweden initiated a long-term monitoring program of multiple habitat types and trophic levels to follow the effects of acidification and recovery of regionally-representative stream ecosystems (e.g. Wiederholm & Johnson 1997). Stream categories consist of (i) sites not deemed to be affected anthropogenic stressors and (ii) sites judged to be stressed by acidification and nutrient enrichment. At present, some 31 streams have been/are sampled annually for water chemistry (often monthly) and riffle macroinvertebrate communities (once in late autumn). In "reference" streams, five replicate macroinvertebrate samples are collected using standardized kick-sampling. In contrast to the sampling protocol of the national stream survey the samples are sorted and identified individually (i.e. not pooled).

To more unequivocally analyze the effects of acidity on macroinvertebrate communities, streams judged to be affected by other anthropogenic stressors were removed from the data set. Consequently, streams affected by agriculture (e.g. > 20 % of the catchment classified as agriculture), urbanization (> 0.1 % of the catchment classified as urban), and liming were removed from the data sets. Sites with a mean pH > 7.5 were also excluded in order to place more focus on the acidity gradient. In addition, for sites sampled in the national surveys, streams were removed if the habitats sampled were not classified as having hard-bottom substratum (i.e. sites scored as predominantly fine, sand or block substrates were removed). For clarity, streams that are considered as reference and acid that were sampled as part of the national stream surveys are referred to here as "national" streams, whilst streams that were sampled as part of the national monitoring program are referred to as "acid-reference" streams.

RESULTS

National survey data

Of the ca. 700 streams sampled in the 1995 and 2000 surveys, 316 sites remained after applying these selection criteria (Fig. 1). About half of the streams (47%) were situated in the Fennoskandia region (Illies ecoregion 22) and had catchments classified for the most part as forest (Table 1). Total phosphorus concentrations and water color were lower in 1995 (15.3 ± 0.7 and 0.127 ± 0.006, respectively) than 2000 (21.2 ± 3.7 and 0.188 ± 0.009). Mean pH and alkalinity was similar between the two surveys; mean pH for 1995 was 6.8 compared to 6.7 for 2000. Although mean pH was similar, the dataset consists of relatively broad gradients in pH and alkalinity (Fig. 2). For example, minimum pH was 4.52 for the streams sampled in 1995 and 4.45 for those sampled in 2000 (maximum pH was 7.5 which may be due to the exclusion of lakes with pH > 7.5). The 10^{th} percentile for alkalinity was 0.077 meq/L in 1995 and 0.055 meq/L in 2000.

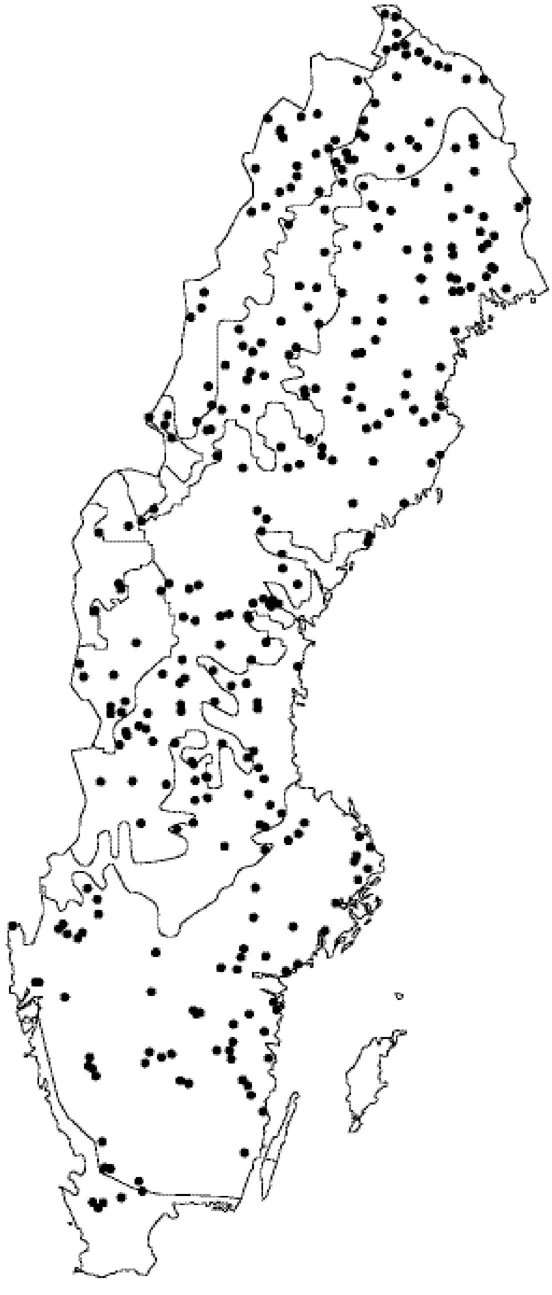

Figure 1. Location of the 316 national streams following pos-stratification of the 1995 and 2000 national stream surveys. Streams are shown as situated in the six major ecoregions.

Table 1. Mean (± 1 SE) of selected water chemistry variables for 316 national streams sampled in the 1995 and 2000 national stream survey.

	1995 & 2000	**1995**	**2000**
Illies Central Plains (%)	23 ± 2.4		
Illies Fennoskandia (%)	47 ± 2.8		
Illies Boreo highlands (%)	30 ± 2.6		
% forest	69 ± 1.4		
% alpine	11.7 ± 1.5		
gravel (score)	1.4 ± 0.04		
fine stone (score)	1.8 ± 0.04		
course stone (score)	1.9 ± 0.04		
pH		6.8 ± 0.02	6.7 ± 0.02
alkalinity/acidity (meq/L)		0.298 ± 0.026	0.247 ± 0.021
ANC (meq/L)		0.406 ± 0.035	0.355 ± 0.022
NO2+NO3-N (µg/L)		129 ± 26	111 ± 18
TP (µg/L)		15.3 ± 0.7	21.2 ± 3.7
water color (absf)		0.127 ± 0.006	0.188 ± 0.009

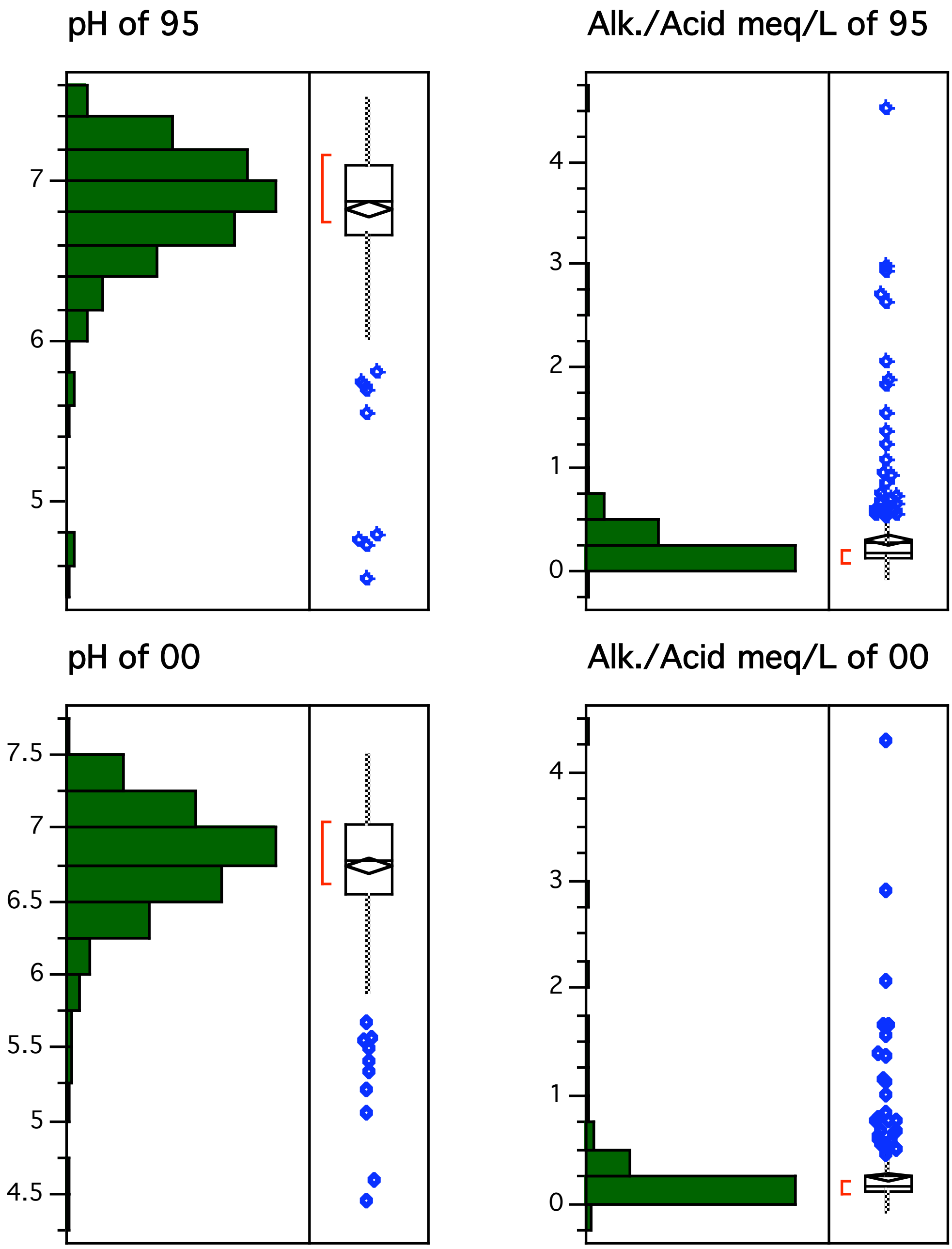

Figure 2. Distribution plots of pH and alkalinity/acidity for 316 national streams sampled in the 1995 and 2000 national stream survey.

Relationships between riffle macroinvertebrate assemblages and acidity

Regression of selected biological metrics (taxon richness and Medin's acid index) showed poor fits with pH, alkalinity and ANC (Fig. 3 and 4), with only the relationship between pH and taxon richness for 2000 found to be significant ($p = 0.035$), but the variation explained was very low (1.4%). Regression of Medin's acid index against the three acidity metrics showed better fits compared to taxon richness. Only one of the six relationships was not significant (Medin's acid index versus alkalinity/acidity, $p = 0.099$). However, similar to taxon richness, the amount of variation explained by pH and buffering capacity was low. For example, the "best" relationship was found between pH and Medin's acid index for 2000 (8% of the variance was explained). Removal of sites with ANC > 1 meq/L improved the fits of several of the relationships, but only marginally. Using the 1995 dataset, pH, alkalinity/acidity and ANC explained 2%, 1.3% and 1.3% of the variance in Medin's acid index, whilst for the 2000 dataset values of 7%, 9.2% and 8.3% were noted.

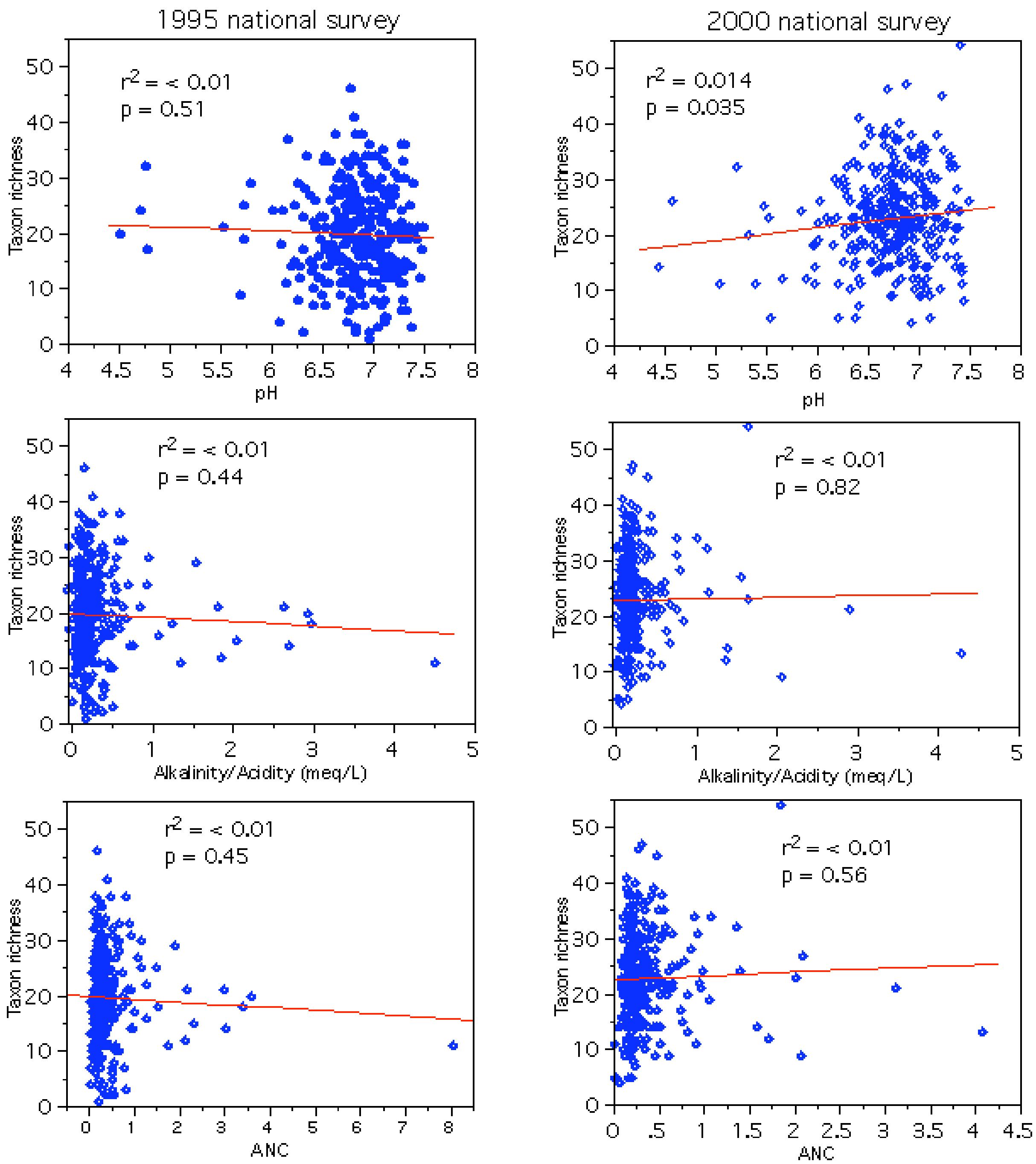

Figure 3. Taxon richness of 316 national streams sampled in the 1995 and 2000 national stream survey plotted against pH, alkalinity/acidity (meq/L) and ANC (meq/L).

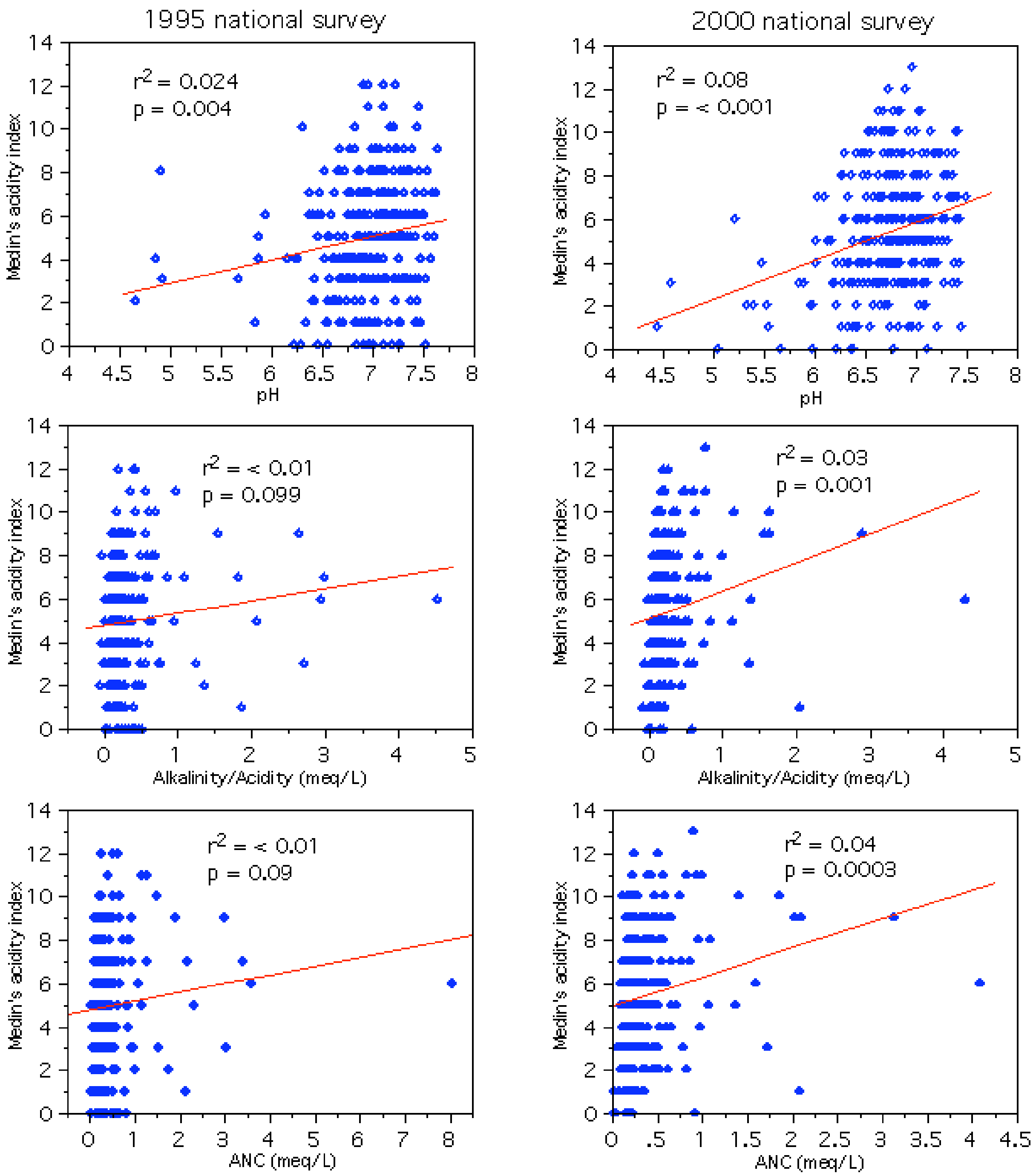

Figure 4. Medin's acid index of 316 national streams sampled in the 1995 and 2000 national stream survey plotted against pH, alkalinity/acidity (meq/L) and ANC (meq/L).

Reference stream data

None of the 31 acid-reference streams were excluded when the pressure criteria for agriculture, urbanization or liming were applied. The reference streams were relatively evenly distributed across the country (Fig. 5). Only data from single years were available for half the streams (n = 15), whereas for 13 streams more than three years of data were available, and three of these streams (Lill-Fämtan, Lommabäcken and Pipbäcken) have been sampled for more than 10 years (Table 2).

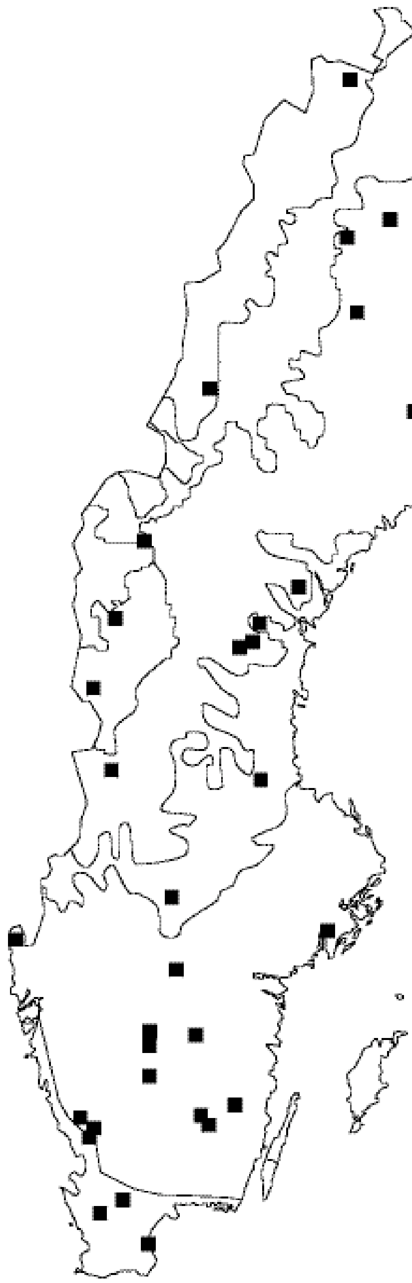

Figure 5. Location of the 31 acid-reference streams by six ecoregions.

Table 2. Thirty-one acid-reference streams sampled as part of the national monitoring program.

Name	X coordinate	Y coordinate	Number of years	Start	End
Alep Uttjajåkkå	739283	163835	4	1997	2002
Bjurbäcken	718265	171875	3	2000	2002
Dammån	632137	147160	1	1997	1997
Domneån Utl. Vättern	641827	139990	1	2001	2001
Ejgstån	654552	123925	1	1997	1997
Fiskonbäcken. v.vid mynn	720990	147270	2	2000	2002
Gnyltån	638065	139975	1	1997	1997
Hornsjöbäcken	697145	157980	1	1997	1997
Hångelån	689815	150920	3	2000	2002
Häradsbäcken	642969	145547	1	2001	2001
Hörlinge	623061	136831	1	1997	1997
Kagghamraån	655640	161440	1	2001	2001
Laxtjärnsbäcken	730224	165025	9	1986	2000
Lill-Fämtan	675032	135400	11	1986	1996
Lillån (Oskarsström)	630695	132775	1	1997	1997
Lillån-Bosgårdsån	631840	133310	1	1997	1997
Lommabäcken Nedre	650920	143244	12	1985	1996
Morån	634570	150290	1	1997	1997
Muddusälven	741419	169012	3	2000	2002
Norrhultsbäcken	633316	146198	1	2001	2001
Pessisjåkka	758311	164144	3	2000	2002
Pipbäcken Nedre	633070	131710	12	1986	1996
Rändan	693301	135878	1	1997	1997
Semlan	702632	139407	2	2000	2002
Skärån. Skäralid	621495	134055	3	2000	2002
Stormyrbäcken	690530	152405	9	1988	1996
Stråfulan	684875	133226	1	1997	1997
Svedån Sved	643455	140114	2	2001	2002
Sörjabäcken (Lillån)	673815	153365	1	1997	1997
Trösälven	659670	142700	3	2000	2002
Viskansbäcken	692688	153260	3	2000	2002

The acid-reference streams were relatively nutrient poor, with a mean TP of 12.5 µg/L, and ranged from brown- to clear-water systems (Table 3). Five streams were very nutrient poor with TP concentrations < 5 µg/L (Stråfulan, Hornsjöbäcken, Semlan, Fiskonbäcken and Pessisjåkka). Water color averaged 0.174 (absf), but one stream was very humic (Dammån) with a water color > 0.600 Relatively broad gradients in pH and buffering capacity were also noted. Mean pH and buffering capacity were 6.6 and 0.295, respectively, however, four streams had a mean pH ≤ 5.0 and alkalinity/acidity < 0 (Lill-Fämtan, Lillån-Bosgårdsån, Lommasjöbäcken and Pipsjöbäcken) (Fig. 6).

Table 3. Selected water chemistry variables of acid-reference streams (n = 31). Values are for all site/year combinations.

	mean ± 1SE	min	max
pH	6.57 ± 0.15	4.28	7.84
Alkalinity/Acidity (meq/L)	0.295 ± 0.085	-0.025	2.61
ANC (µeq/L)	408 ± 93	-11.5	2765
NO2+NO3-N (µg/L)	202 ± 64	3	1735
TP (µg/L)	12.5 ± 2.1	2	53
water color (absf)	0.174 ± 0.023	0.016	0.634

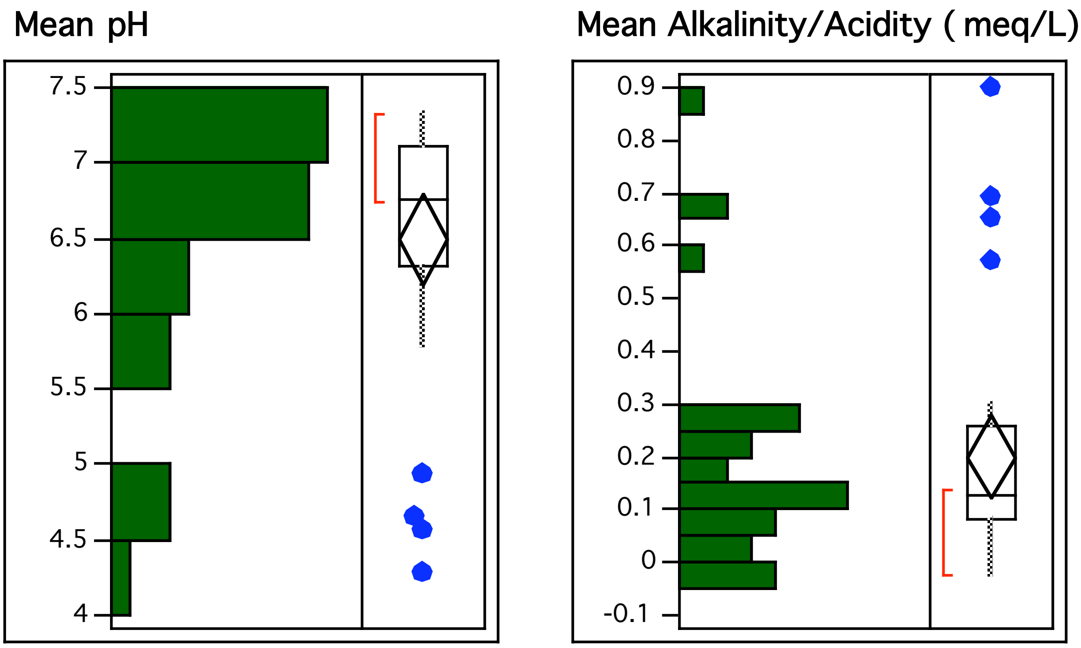

Figure 6. Distribution plots of pH and alkalinity/acidity for31 acid-reference streams.

Relationships between riffle macroinvertebrate assemblages and acidity

Stepwise regression was run using data from the acid-reference streams to determine the best predictors of taxon richness, diversity and Medin's acid index. The majority of the water chemistry variables selected in the models were indicative of acidity. This finding is not too surprising, since the effects of other pressures in these systems (such as agriculture) are low. Regression of the three biological metrics and selected stream variables showed that minimum pH was the best predictor of taxon richness ($r^2 = 0.33$), mean Mg concentration was the best predictor of Shannon diversity ($r^2 = 0.19$), and mean ANC was the best predictor of Medin's acid index ($r^2 = 0.52$) (Table 4). The three models explained from 38% (Shannon diversity) to 83% of the variability in index values among the acid-reference streams. The finding that ANC was a good predictor of Medin's acid index (ANC explained 52% of the among-stream variability) was expected as this metric weights acid sensitive/tolerant taxa differently.

Re-running the analyses with only pH, alkalinity/acidity and ANC showed that minimum pH was the only variable selected as the best predictor of taxon richness (r^2 = 0.33). For Shannon diversity two of the eight variables were selected (r^2 values for minimum pH = 0.14 and for median pH = 0.06). For Medin's acid index five variables were selected and these variables explained 74% of the among-stream variability. The first variable selected was mean ANC (52%), followed by median alkalinity/acidity (10%), minimum pH (8.2%), minimum alkalinity/acidity (2.2%), and median pH (1.7%)

Table 4. Stepwise regression of three biological metrics and geographic position and selected water chemistry variables using data from 31 acid-reference streams. R^2 values are given and those in bold were the first variables selected in the forward selection.

Variable	Taxon richness	Shannon diversity	Medin's acid index
latitude			0.013
longitude			
mean pH			
median pH			
min pH	**0.33**	0.05	0.08
mean conductivity			
mean Ca			0.02
mean Mg		**0.19**	0.07
mean alkalinity/acidity		0.08	0.10
median alkalinity/acidity			
min alkalinity/acidity			
mean ANC			**0.52**
median ANC		0.07	
min ANC			
mean SO4			
mean Cl			
mean NO2+NO3	0.03		0.03
mean TN			
mean TP			
mean water color	0.13		
model r^2	*0.49*	*0.38*	*0.83*

** Verkaån was exclude due to high alkalinity*

Linear regression models were also done for each of the three biological metrics and metrics of acidity (Table 5) to determine if mean values could function as surrogate indicators of extreme (i.e. minimum) values. All regression models of Medin's acid index against acidity metrics were highly significant ($p < 0.001$). In contrast, taxon richness was significantly related to six of the nine measures of acidity and Shannon diversity with only one acidity metric (minimum pH). Comparison of mean, median and minimum values for pH, alkalinity/acidity and ANC showed that minimum values generally explained slightly more the variance in taxon richness and Shannon diversity, whilst mean ANC was a slightly better predictor for Medin's acid index. Differences were, however, small. For example, minimum pH explained 3%, 4% and 1% more of the variability in taxon richness, Shannon diversity and Medin's acid index than mean pH.

Table 5. Linear regression of three biological metrics and mean, median and minimum values of pH, alkalinity/acidity and ANC using data from 31 acid-reference streams†. R^2 with associated p-values.

Variable	Taxon richness	Shannon diversity	Medin's acid index
mean pH	0.29 **	0.09	0.46 ***
median pH	0.28 **	0.08	0.45 ***
min pH	0.32 ***	0.13 *	0.47 ***
mean alkalinity/acidity	0.09	0.00	0.41 ***
median alkalinity/acidity	0.07	0.00	0.38 ***
min alkalinity/acidity	0.08	0.02	0.37 ***
mean ANC	0.16 *	0.04	0.50 ***
median ANC	0.16 *	0.04	0.49 ***
min ANC	0.16 *	0.06	0.47 ***

*p < 0.05; ** p < 0.001; *** p < 0.001
† *Verkaån was exclude due to high alkalinity*

SETTING CLASS BOUNDARIES

Plots of the three biological metrics (taxon richness, Shannon diversity and Medin's acid index) against the three metrics of acidity (pH, alkalinity/acidity, ANC) showed the expected funnel-shaped or dose-response related response for several of the relationships. In particular, taxon richness and Medin's acid index showed funnel-shaped responses. Plots of data from the national stream survey were much more variable (Fig. 7 and 8) than similar plots using acid-reference streams. This finding could be due to the single water chemistry samples versus the more integrated response of the macroinvertebrate communities. For example, although several of the acid-reference streams were sampled only "once" this one sample generally consisted of several within year samples. Similar plots of the three biological metrics against the three acidity metrics were done using all stream-year combinations (Fig. 9 and 10). As no large differences were noted between the use of mean, minimum or median values of pH, alkalinity/acidity and ANC and the biological response variables tested here are plotted against mean values of pH, alkalinity/acidity and ANC.

The five class boundaries used for pH and the three or four class boundaries used for alkalinity/acidity and ANC are taken from Johnson et al. (2004). For pH the five classes used are 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). For buffering capacity three classes were used for alkalinity/acidity and four cut-levels were used for classifying ANC. For alkalinity/acidity the three classes are < 0.02 meq/L (class 1), 0.020 – 0.10 me/L (class 2) and > 0.10 meq/L (class 3), with an extra class (0.020 – 0.050) used for ANC.

As mentioned, scatter plots of data from the national stream survey were more variable than those using the acid-reference stream data. The three biological metrics showed clear responses to pH, in particular the relationships found for the acid-reference streams. Sites situated in class 1 (pH < 5.0) often showed low variability compared to the other four classes. For example, Medin's acid index was ≤ 3 for both the national survey and reference stream data. In contrast, class 4 (pH 6.2 – 6.8) and class 5 (pH > 6.8) showed considerable variation, with the majority of the sites having a Medin's acid index score > 6. A score of 6.0 is considered as the cutoff below which sites are deemed to be showing the effects of acid stress (Anonymous 1999). Although none of the acid-reference streams had pH between 5 and 5.5, seven of the national survey sites occurred in this interval, five of which had scores ≤ 2. These findings indicate that classes 1 and 2 are clearly acid, whereas sites in class 3 show slightly higher variability.

For buffering capacity, the three or four classes seemed to capture the variability in Medin's acid score. Using alkalinity/acidity, the majority of sites had acid scores < 3, and no sites had scores < 6. The interval between 0.02 and 0.1 meq/L showed higher variability, with acid scores mostly between 3 and 10 (national stream survey data) or 3 and 12 (acid-reference stream data). Sites with alkalinity/acidity > 0.10 meq/L showed high variability, with many sites having acid scores > 6 (acid-reference stream data). For ANC a similar pattern was noted. The extra class of ANC between 20 and 50 µeq/L seems unwarranted according to the acid-reference stream data. Only two streams had ANC values in this range and both had scores ≤ 1. For the national stream survey data three stream sites had ANC values between 20 and 50 µeq/L and two of these had acid score values ≤ 1 whereas the third had a score of 3.

In summary, plots of the three biological metrics showed significant relationships with in-stream measures of acidity. These data due not imply the effects of human-induced change, as no effort has been made to determine if these sites are anthropogenically affected by acidification or naturally acid. Comparison of the stream biological-acidity gradients with those studied by Johnson et al. (2004) suggest that streams display more variability across the acidity gradients studies. Nevertheless, although classifications were not as robust as those determined for lake littoral macroinvertebrate assemblages, the classifications are similar. For pH, the first three classes (i.e. class 1 to 3) showed gradual increases in taxon richness and Medin's acid index. The latter two classes (class 4 and 5), on the other hand, were not as clearly defined (higher variability) as those using lake assemblages (Johnson et al. 2004). Regarding alkalinity/acidity and ANC, again the highest class (class 3 for alkalinity and class 4 for ANC) showed high among-site variability in all three biological metrics. Use of four classes for ANC, or the "extra" class of ANC between 20 and 50 µeq/L (class 2) is not justifiable with these data, but may be warranted if classification of lake assemblages are to use four classes.

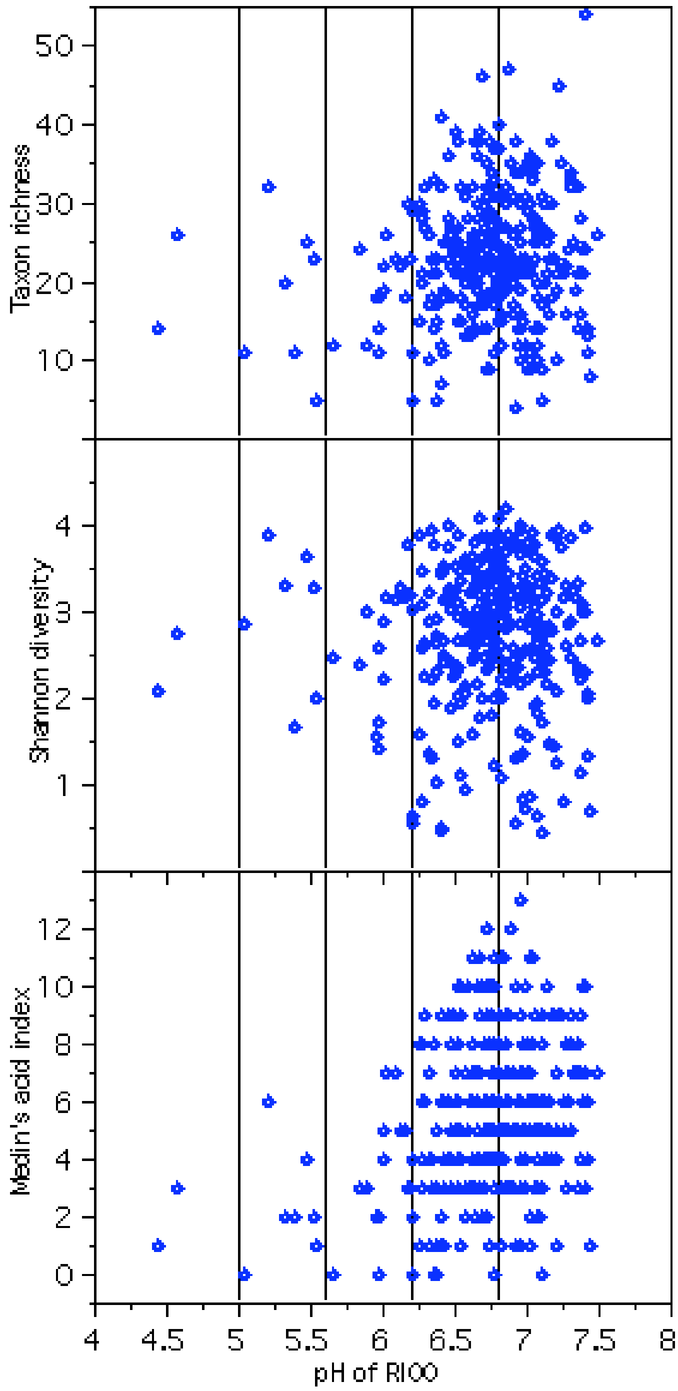

Figure 7. Scatter plots of taxon richness, Shannon diversity and Medin's acid index against pH for 316 national streams sampled in the 2000 national stream survey. Vertical lines show the class boundaries defined by Johnson et al. 2004.

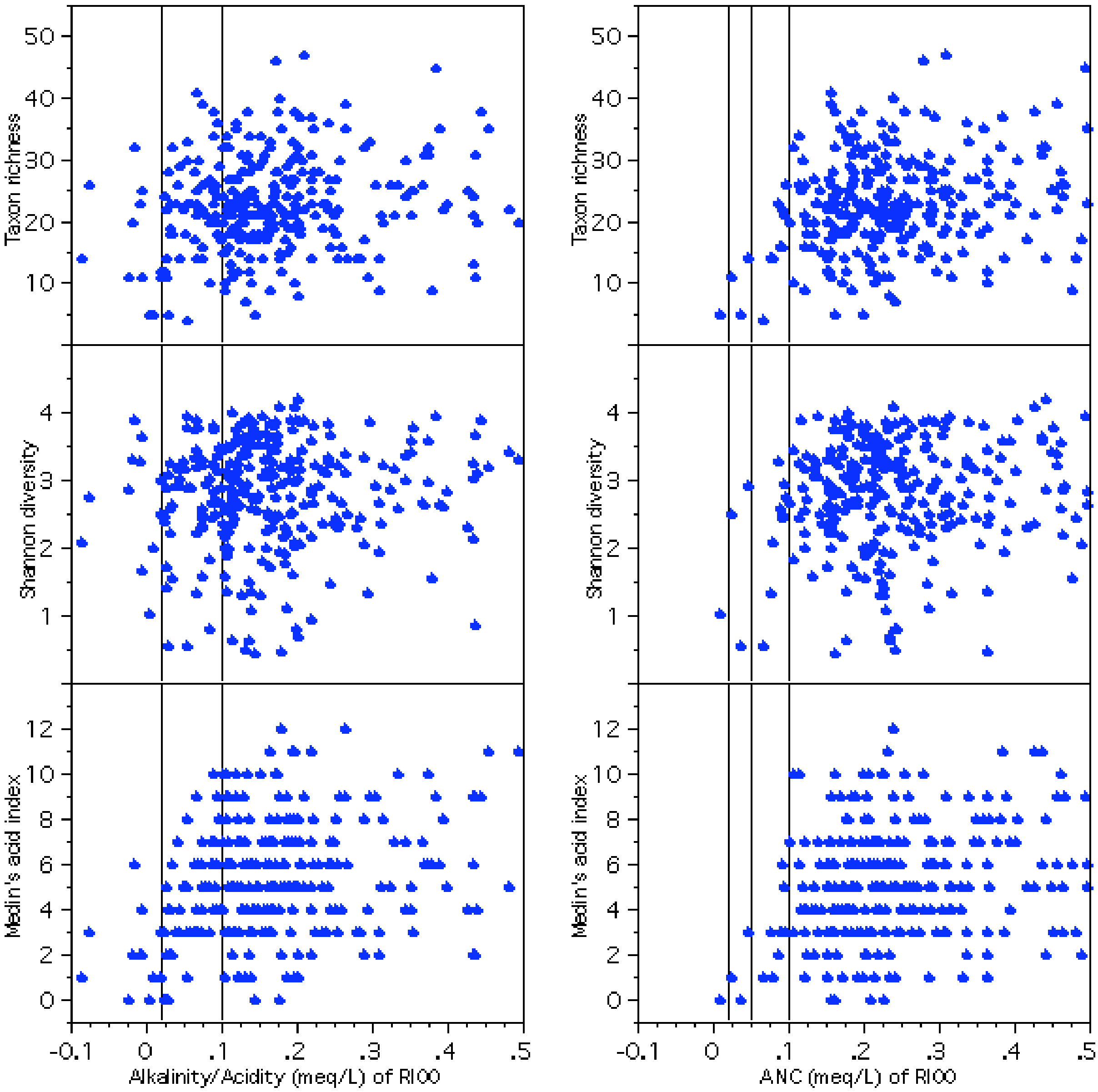

Figure 8 Scatter plots of taxon richness, Shannon diversity and Medin's acid index against alkalinity/acidify (meq/L) and ANC (meq/L) for 316 national streams sampled in the 2000 national stream survey. Vertical lines show the class boundaries defined by Johnson et al. 2004.

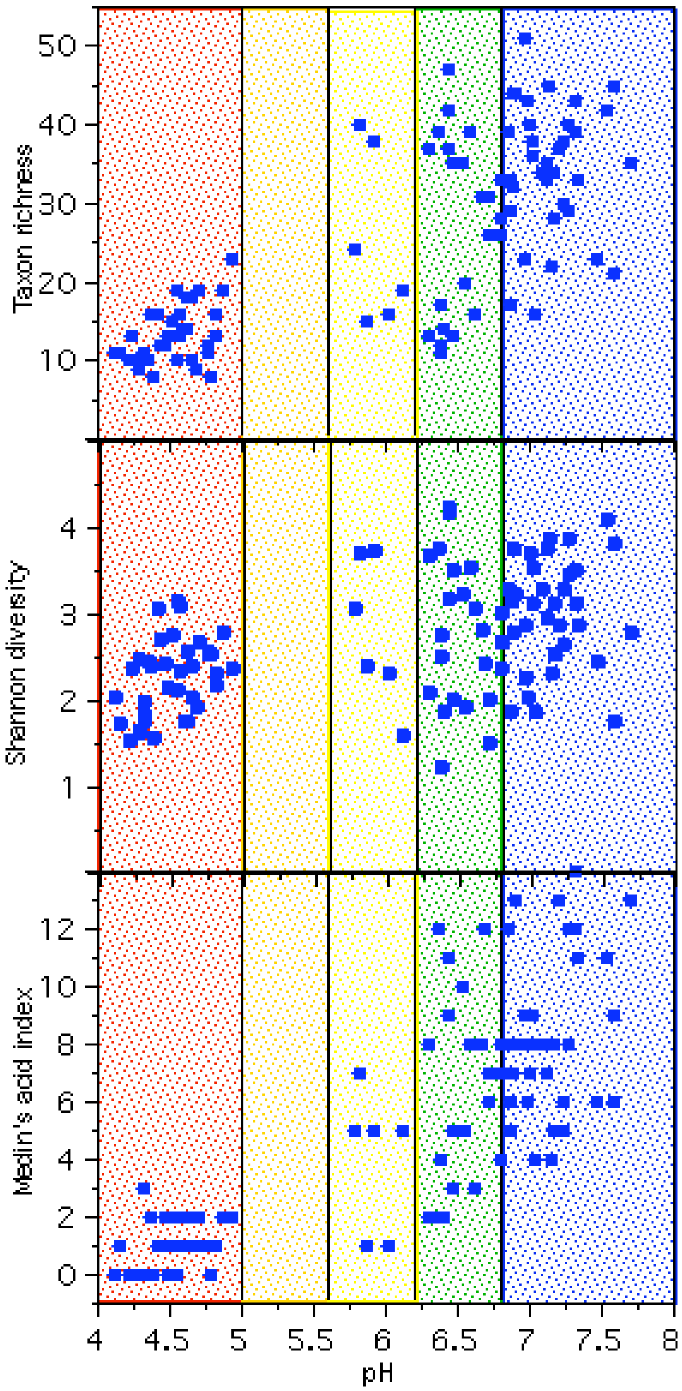

Figure 9. Scatter plots of taxon richness, Shannon diversity and Medin's acid index against pH for 31 acid-reference streams (see Table 2). Vertical lines show the class boundaries defined by Johnson et al. 2004.

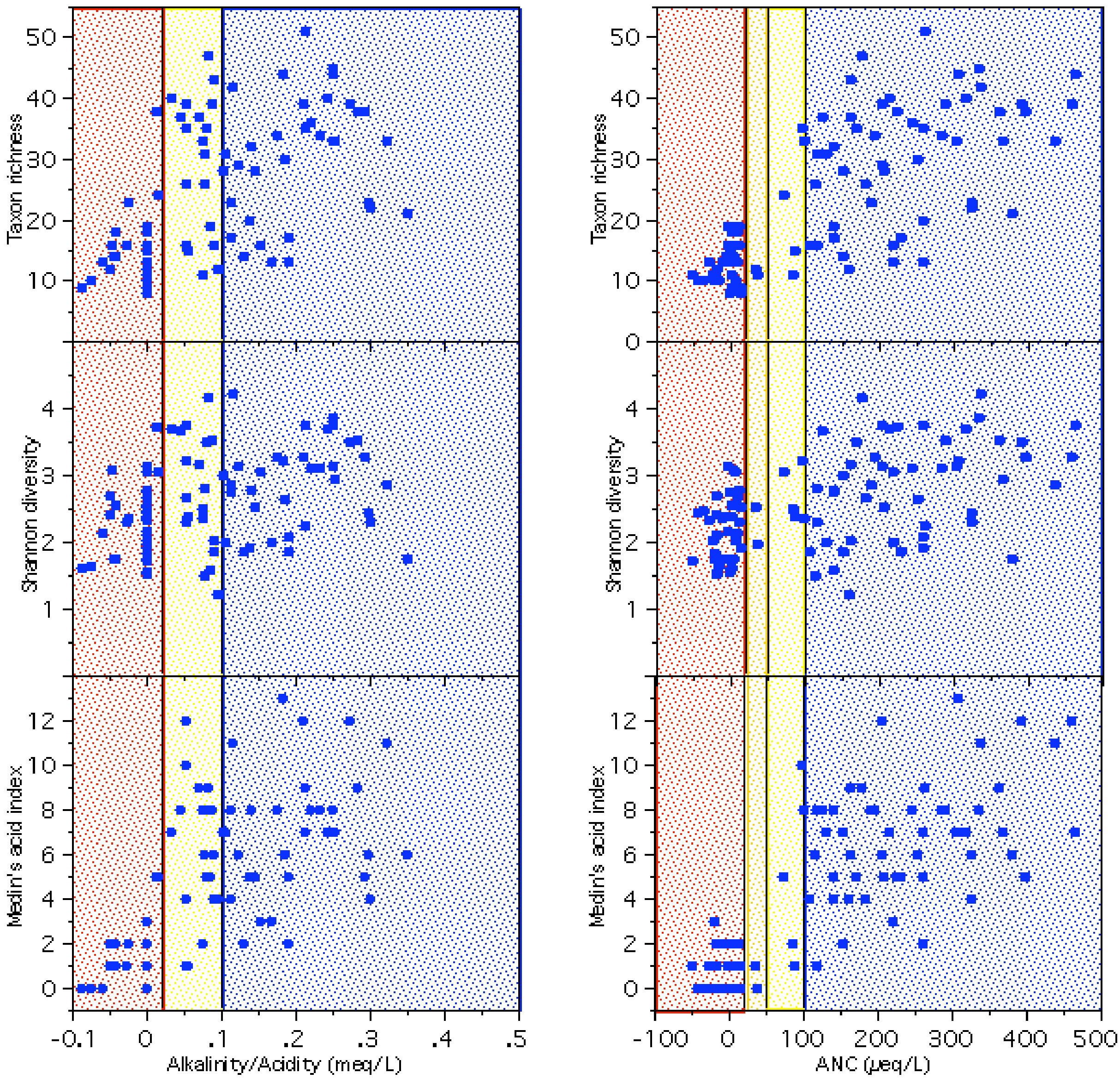

Figure 10. Scatter plots of taxon richness, Shannon diversity and Medin's acid index against alkalinity/acidity (meq/L) and ANC (µeq/L) for 31 acid-reference streams (see Table 2). Vertical lines show the class boundaries defined by Johnson et al. 2004.

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