3 Methods

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3.1 Water Chemistry

Sampling and analytical methodologies conform to those outlined by Patrick *et al.* (1991) while the approach to Analytical Quality Control (AQC) is provided by Patrick *et al.* (1995). UKAWMN laboratories participate in an AQC programme run by the Water Research Centre, Medmenham, and results are provided to the UKAWMN in annual internal reports which are available on request. The programme has found a high standard of accuracy for all the major determinands analysed in this report. Small discrepancies have been observed in the measurement of Al which seems to result from the differing procedures used in the separation of the labile fraction. These differences were not detected until well into the monitoring period, and it is now considered more appropriate, for monitoring purposes, to maintain internal consistency rather than to improve inter-laboratory consistency.

3.1.1 Data screening

Chemical data were screened to remove erroneous values prior to analysis. Charge balance errors were calculated as:

$$CB\ (\%) = \left[\frac{\sum Base\ Cations - \sum Acid\ Anions - Alkalinity}{\sum Base\ Cations + \sum Acid\ Anions + Alkalinity}\right] *100\%$$

where all ion concentrations are in μeq l^{-1}. Charges of aluminium and organic anion species could not be quantified, and the charge balance is therefore approximate; however in general the error should

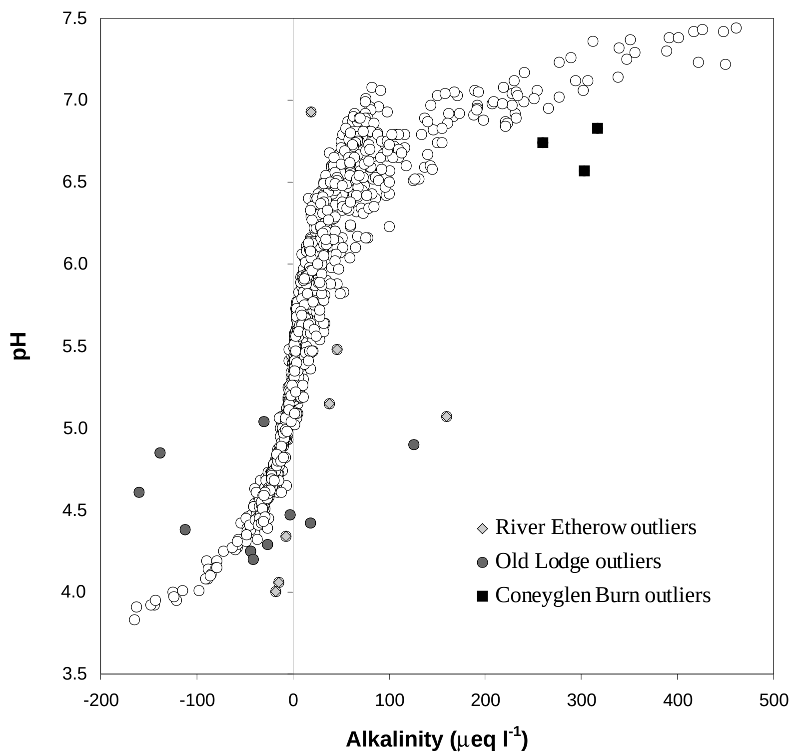

Figure 3.1

pH vs Alkalinity for the full UKAWMN dataset

be close to zero. Errors of greater than 10% were therefore taken to indicate a possible analytical error. Where this error was associated with a clear outlying value (defined as a measurement outside the range of all other measurements at that site) this value was discarded. This procedure led to the removal of one or more determinand value in a total of 14 samples. Samples with charge balance errors greater than 10% but no clear outliers were retained in the dataset.

Data were also screened on the basis of the relationship between alkalinity and pH. These determinands are closely related through carbonate equilibria, and should plot on a sigma curve (Munson & Gherini, 1991). A plot of pH vs. alkalinity for the entire UKAWMN dataset shows the expected relationship (Figure 3.1), but a small number of outliers are observed for Coneyglen Burn, the River Etherow and Old Lodge. Coneyglen Burn has very high DOC concentrations (Section 4.22) and the low pH relative to alkalinity in these samples may be explained by weak organic acidity (Munson & Gherini, 1991). However the seven outlying data points at the Etherow and ten at Old Lodge were considered to be outside the acceptable range and were removed.

Finally, wider problems were identified for unstable determinand data at the River Etherow and Old Lodge during the first three years of monitoring. At this time, unstable determinand analysis for the two sites was undertaken at local laboratories, but from April 1991 this was transferred to SOAFD, Pitlochry. Time series data for alkalinity at the Etherow, and for alkalinity, pH and NO_3 at Old Lodge show markedly higher scatter prior to the laboratory change (Figure 3.2), suggesting a poor level of analytical precision at this time. All samples at both sites failing to conform to the expected pH/alkalinity relationship were also collected during this period. The first three years of data for alkalinity at both sites, and for pH and NO_3 at Old Lodge, were therefore discarded.

3.1.2 Statistical analyses

Changes in water chemistry over the ten years were assessed using two parallel trend detection methods. The Seasonal Kendall Test (SKT) is a non-parametric procedure for detecting monotonic changes over time, developed by Hirsch *et al.* (1982). SKT has been identified by Taylor & Loftis (1989) as the most suitable non-parametric trend identification technique for water quality data containing seasonal variations, and was used in the UKAWMN five year analysis (Patrick *et al.*, 1995). Values are grouped into seasonal blocks (months for streams and quarters for lakes) and tested for trends using a ranking procedure. SKT is robust with respect to non-normality, missing or censored data and seasonality, but in its original form is sensitive to serial correlation. The method used here is therefore a modified version described by Hirsch & Slack (1984), which is robust providing auto-correlation is less than 0.6 and records extend for more than five years. Slopes associated with trends found to be significant at the 95% level were then estimated according to the method of Sen (1968), as the median of all between-year differences within each seasonal block.

The second trend detection procedure used was simple linear regression, with a significance test adapted to take account of non-normality in the distribution of sampling times. Having first computed the r^2 value for the data arranged in chronological order, the procedure was then repeated 8000 times for data arranged in random order. Significance was determined as the position of the initial r^2 value within the distribution of all r^2 values, with a 95% significance level (i.e. the initial r^2 in the top 5% of all calculated r^2 values) taken as a threshold for trend identification. This method was used in the UKAWMN five year analysis, and is described by Patrick *et al.* (1995). It has also been used in a trend analysis of Scottish surface waters by Harriman *et al.* (1995a).

Of the two trend detection methods, SKT is particularly effective where seasonality is present, but may be more conservative in detecting trends where it is not. SKT also tends to identify changes more effectively where these occur steadily over time, whereas regression is more likely to show a significant trend resulting from a step change. Both methods are however limited to the detection of monotonic trends, whereas solute concentrations may fluctuate over

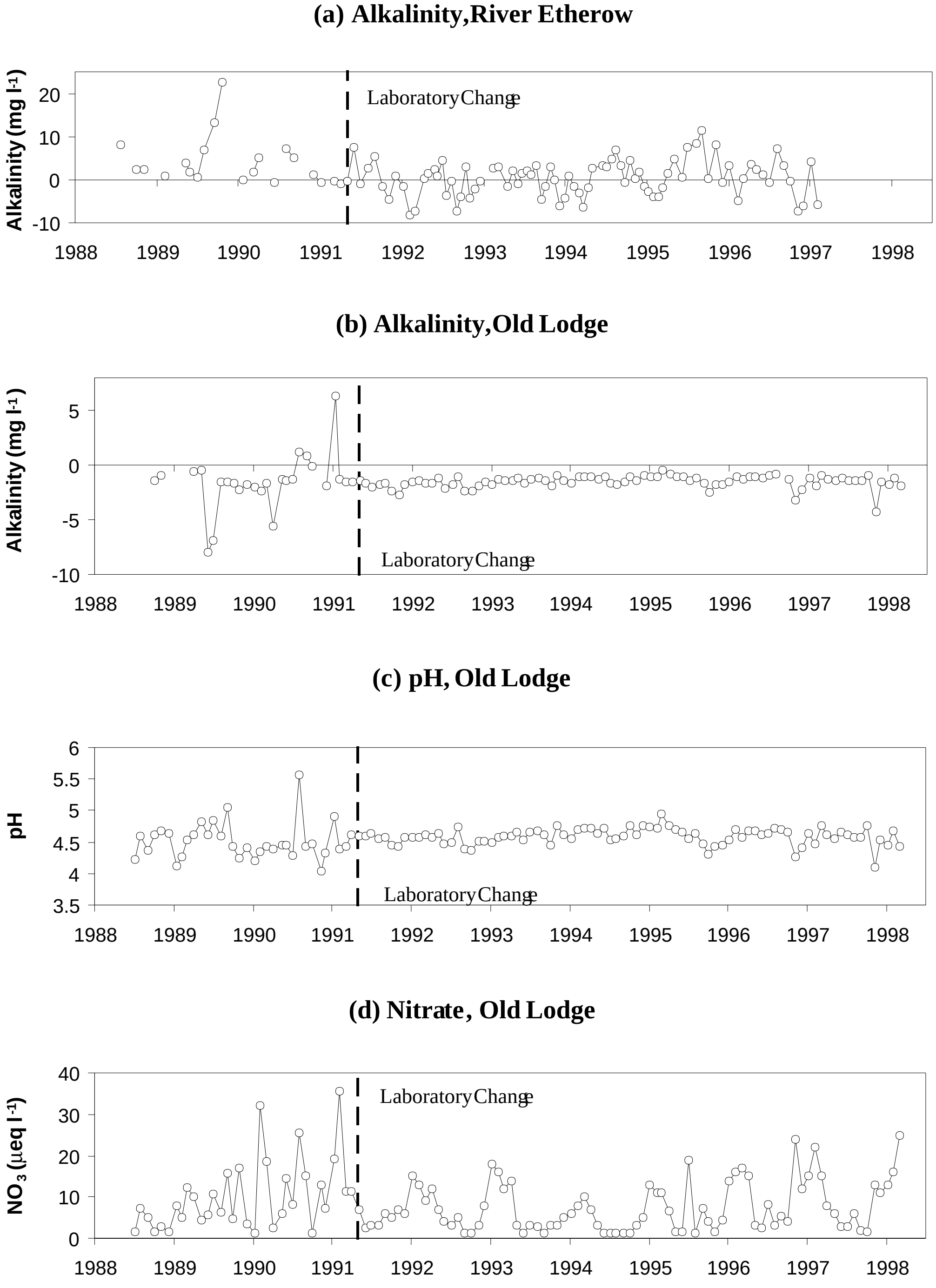

Figure 3.2
Time series data for alkalinity for the River Etherow (a) and Old Lodge (b), and pH and NO_3 for Old Lodge. Dotted line indicates time of laboratory change

time in response to anthropogenic or natural changes. Robson & Neal (1996) have shown that SKT can potentially identify a significant trend in data containing only a long term cyclical signal. In order to identify more complex variations in the dataset, therefore, the Splus LOESS curve-fitting function was used (Cleveland, 1979; Becker *et al.,* 1988). The procedure provides robust smoothing using a local quadratic polynomial fit. For each date value, a weighting procedure is applied to nearby points, and a local curve fitted using a least squares optimisation. LOESS curves were added to all time series plots other than those where concentrations were consistently at or close to detection limits.

Trend analyses were undertaken for pH, alkalinity, total sulphate and non-marine sulphate, nitrate, chloride, dissolved organic carbon, the four major base cations (calcium, magnesium, sodium and potassium), total aluminium, and labile and non-labile aluminium fractions. Other measured chemical constituents were generally present at low concentrations, often below detection limits, and were therefore unsuitable for trend assessment.

■ 3.2 Biology

Sampling, analysis and quality control procedures conform to the protocols provided by Patrick *et al*. (1991) and Patrick *et al*. (1995). Following recommendations by Patrick *et al.* (1995), the aquatic macrophyte survey frequency for lakes was reduced from annual to once every two years, from 1993 onwards. Determination of fish condition factor has occasionally been prevented at a few sites by the failure of weighing apparatus in the field. Steps have now been taken to ensure this problem does not recur in the future.

3.2.1 Data screening

Data entry of multi-species records on the UKAWMN database (i.e. for epilithic diatoms, aquatic macrophytes and macroinvertebrates) involves the input of alphanumeric species codes according to recognised species coding systems. Errors could occur in the entry of a species codes or other data, such as abundance measurements. Species abundance data for the multi-variate datasets of each site were therefore plotted graphically, complete with full species names, and scrutinised visually to identify obvious outliers, or apparently missing data points. For fish data, all records of weight which were >4 times the standard deviation from the mean were omitted from the dataset. In addition, fish length was plotted against fish width for each site and outliers were identified by visual inspection. Where outliers were identified, original record-sheets were re-consulted and amendments made to the database where necessary.

3.2.2 Statistical analyses

<u>Epilithic diatom and macroinvertebrate analyses</u>

Epilithic diatom data consist of the frequency of all taxa occurring in a count of 300 diatom valves for each sample collected. For most sites, three replicate samples are collected in late summer to early autumn (i.e. July-September). Macroinvertebrate data consist of the frequency of all taxa collected in each one minute littoral kick sample. Sampling is conducted from April-May. From 1988-1991, three replicates were collected from each site, but since 1992 this has been increased to five. In order to reduce errors of mis-identification, some species or genera that are difficult to identify have been combined into a higher taxonomical level. The genus *Nemoura* has been spilt into two easily identifiable groups: *Nemoura* spp. 1 (long legged - including *N.cinerea*, *N. dubitans* and *N. avicularis*) and Nemoura spp. 2 (short legged - *N. cambrica* and *N. erratica*). Taxa corresponding to the Heteroptera Gerromorpha have been ignored since these are semi-aquatic and live primarily on the water surface.

The data used in all analyses consisted of all taxa identified to species level or lower. For macroinvertebrates, the acid tolerant mayfly family Leptophlebiidae, and the stonefly groups *Nemoura* sp. 1 and *Nemoura* spp. 2, were also included as a pseudo-species. Data were converted to percentages and transformed by log-ratio centring prior to Redundancy Analysis (RDA) and Principal Components Analysis (PCA). All analyses were performed using the program CANOCO 3.12a (ter Braak, 1988, 1990).

Statistical analyses followed that outlined by Patrick *et al*. (1995). Data were tested for time trends using RDA, a form of PCA in which components are constrained to be linear combinations of explanatory variables (Jongman *et al.*, 1987; ter Braak & Prentice, 1988). Linear methods were deemed appropriate after detrended canonical correspondence analysis (DCCA) demonstrated that all datasets had short time constrained gradients (<3 standard deviation units).

Two forms of RDA were performed:

i) Sample year was coded as multiple 'dummy' variables. In this analysis the sum of constrained eigenvalues represents the proportion of the total variance in the species data which can be explained by differences between years. The remaining proportion therefore represents the variance between replicate samples from the same year.

ii) Sample year was coded as a single variable. Here, the sum of constrained eigenvalues represents the proportion of the total variance which may be explained by a linear trend (λ_1 RDA).

Statistical significance was assessed using two forms of Monte Carlo permutation test (Manly, 1991; Potvin & Roff, 1993) with 999 permutations of samples. The unrestricted permutation test assumes independence among observations and for time-series data this assumption is often invalid. The restricted permutation test restricts the range of possible permutations to preserve the autocorrelation structure of the data (ter Braak, 1990). Results for both tests are presented in the site summaries of Chapter 4 but conclusions are only based on the restricted test, which appears to be generally more conservative (i.e. fewer trends are deemed significant by this method).

PCA was also conducted for all multi-species datasets. The first axis of PCA provides the maximum variance which can be explained by a single constraining variable (λ_1PCA). The ratio of (λ_1RDA) to (λ_1PCA) therefore provides an indication of the strength of any time trend relative to random and unmodelled effects. Hill's N_2 is a measure of the 'effective' number of species in a sample and allows between-site comparisons.

In Chapter 4, optimal pH values are provided for the diatom species referred to in the summary text. These values are based on statistical analysis of diatom species representation in surface sediments taken from the 167 lakes of the SWAP dataset (Stevenson *et al.*, 1991).

<u>Fish analysis</u>

Data considered in this report are for trout (*Salmo trutta*) and, for two sites Atlantic salmon (*S. salar*), caught by electro-fishing of stream sites and the outflow streams of lake sites. Density estimates are based upon the catch data from three individual reaches fished at each site. Reach population numbers are estimated from a series of constant-effort removal fishings using the Exact Maximum Likelihood method (Carl & Strub, 1978). A chi-square test is used to assess the validity of the assumption that a constant proportion of the fish population are caught and removed by each successive fishing.

All individuals are weighed and their lengths measured, and these data are used to calculate condition factor (CF):

$$CF = \text{weight} / \text{length}^3$$

Three parameters (density, mean condition factor, and the coefficient of variation of the condition factor) for two age classes, (0+, i.e. less than one year old; and >0+, i.e. greater than one year old) have been subject to linear regression analysis to test for time trends. All parameters are presented as time series plots in Chapter 4. However, time trends have almost invariably been insignificant and have not been included in the trend statistic data tables in the site summaries. Significant trends have been reported in the summary text when observed.

To compliment the electro-fishing surveys, a standard habitat assessment (HABSCORE III - HQS) was conducted at each reach. This method was originally developed by the Welsh Water Authority to predict fish populations and is based on empirical statistical models. The HQS gives a predicted density under 'pristine' conditions. By comparing observed fish density with HQS it should be possible to determine whether observed trends are due to changes in habitat or some other limiting factor such as acidity. HQS should also provide an indication of whether densities observed at UKAWMN sites are lower than would be expected were they not acidified. HQS values for most sites show considerable inter-annual variability over the monitoring period, and since few linear trends have been identified in the trout population data HQS data have not been systematically reported in Chapter 4. However, mean HQS data are considered in Section 7.1.3.

<u>Aquatic macrophyte analysis</u>

For lakes, relative species abundance was determined on a five point scale (i.e. comparable to the DAFOR scoring system (e.g. Palmer *et al*., 1992), following shoreline survey, shore transects and deep water grapnel trawls, as follows:

1. rare/infrequent
2. occasional but not abundant
3. widespread but not abundant
4. locally abundant
5. widespread and abundant

For streams, total macrophyte cover was estimated for each 5 metre section of a 50 m survey stretch, and each was then partitioned into proportional species abundance, to provide percentage cover for each species. Data analysed for this report are the mean species cover estimates for the 50 m stretch.

Data analysis follows that for epilithic diatoms and macroinvertebrates described above. Since replicate samples are not taken, RDA to assess the proportion of between-year and within-year variation was not performed. For lakes, raw abundance data were used (i.e. without transformation), while for streams, percentage cover data were log-transformed prior to RDA and PCA. PCA and RDA were based on a species covariance matrix.

3.2.3 Graphical and tabular presentation of data

Data presented graphically in the site summaries (Chapter 4) represent the following:

i) Epilithic diatoms. Percentage frequency of all taxa occurring at over 2% relative abundance in any one sample (for the site). Data for all replicate samples are plotted. Species are sorted from left to right in order of the year of their maximum occurrence. This provides a diagonal structure to the plot if there is any time trend.

ii) Macroinvertebrates. Percentage frequency of all taxa recorded in all replicate samples from the site. In addition, the total number of individuals recorded in each sample. These data are sorted taxonomically, so that species of common Genus, Family etc., cluster together.

iii) Trout.

a) Density of 0+ (new recruits) and >0+ (more than one year old) fish for the site;

b) Mean condition factor (CF) and its coefficient of variation for 0+ and >0+ fish; c) Length frequency histograms (Peterson graphs) to illustrate the population structure at each site for each year.

iv) Sediment traps. Sediment traps have been deployed at all UKAWMN lakes since 1991. Problems with trap retrieval were encountered at some sites during the first two years but since then samples have been collected from most sites in most years. Graphs represent the following:

a) Diatoms. Percentage frequency of all diatom taxa occurring at over 2% relative abundance in any one sample (for the site). Only one sample is collected per year.

b) Spheroidal carbonaceous particles (SCPs). SCPs are derived from high temperature fossil fuel combustion (Rose *et. al*, 1995). Palaeolimnological work has shown similar historical patterns of increase in the carbonaceous particle flux to the sediments of all UKAWMN lakes over the last century or more (Patrick *et al*., 1995), with peak values occurring in the mid- to late 1970s. The recent introduction of emission controls have resulted in a decline in particle fluxes to sediments (Rose *et. al*, 1995). Data are provided for SCPs as numbers of particles per trap per day. Calculated fluxes show high inter-annual variability at most sites but there is no evidence for a decline in flux over the period of trap deployment at any site (Rose pers. comm.). Results have not been discussed in the site summaries (Chapter 4).

Aquatic macrophyte data are presented in tabular format:

Certain species which have been associated in ecological literature with particular levels of acidity (e.g. Farmer, 1990) have been ascribed 'indicator' status denoted by superscripts in the species tables of Chapter 4. For lakes these range from:

[1] - extremely intolerant of acid conditions and rarely found in lakes with mean pH below 5.6;
[2] - characteristic of mildly acid lakes but rarely found in waters with mean below pH 5.2;
[3] - species tolerant of waters below pH 5.2 but rarely found in very acid lakes;
[4] - acidophilic species which tend to dominate waters with mean pH below 4.5.

For streams:
[1] - characteristic of well buffered streams prone to only occasional acid episodes;
[2] - characteristic of well buffered to mildly acid streams;
[3] - characteristic of permanently acid streams.