Environment Agency

Evidence

A WFD compatible approach to assess acidification using diatoms in UK and Irish rivers

Report – SC070034/TR2

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Miranda Kavanagh

Director of Evidence

Executive summary

There are two reasons why there are no suitable metrics for assessment of pH using diatoms for the Water Framework Directive (WFD): no current metric addresses the issue of ‘expected’ values of metrics, nor do any provide an ecological basis for setting quality status boundaries. The former issue is particularly significant as it is important that ecological status assessments can distinguish those water bodies that have been acidified by human causes from those that are naturally acid. An ecological rationale for setting boundaries is also important as the WFD provides ecological criteria by which ecological status should be assessed. The method described here will sit alongside the diatom metric that currently assesses the extent of eutrophication.

The developed model was validated using time-series data from a number of well-studied catchments. In most cases the predicted status class agreed well with the status derived from chemical and invertebrate analyses. Where there was disagreement this could be attributed to the episodic nature of the sites. Diatoms can respond to changes in water chemistry in a matter of days, and diatoms can exhibit different responses to chronic and episodic acidification. Given the rapid temporal response of diatoms to changes in chemistry it is clearly important to sample at the appropriate time of year to encompass any episodes of low pH and acid neutralising capacity (ANC) during periods of high flow. If appropriate sampling can be achieved then the new Diatom Acidification Metric (DAM) provides a relatively simple but effective way of assessing the ecological status of UK soft-waters.

Acknowledgements

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Contents

1 Introduction

There is a long history of research on the response of diatoms to pH, from pioneering studies of Hustedt (1937–1939), through palaeoecological studies on the causes of lake acidification (Renberg and Hellberg 1982, Flower and Battarbee 1983, Kreiser *et al*. 1990, Jones *et al*. 1993), to studies of recovery and the effects of remediation strategies (Flower *et al*. 1990, Juggins *et al*. 1996, Lancaster *et al*. 1996). The focus of this research has been primarily on lakes, where the relationship between diatom assemblages and pH can be modelled with a precision of approximately $\pm$ 0.3 pH units (Birks *et al*. 1990).

This work played an important role in both Europe and North America in highlighting the role of acid deposition in causing environmental change (Battarbee *et al*. 2010) and, ultimately, for shaping government policy. It was one of a number of factors that demonstrated the harmful effects of human activities on ecology and ecological services. The need to recognise situations where human activities had driven deleterious ecological changes led to the development of the Water Framework Directive (WFD: European Union 2000).

A core concept of the WFD is that ecological status (defined as 'an expression of the structure and functioning of aquatic ecosystems', WFD Article 2) is measured by reference to the condition of an ecosystem that would prevail in the absence of significant human disturbances. The magnitude of any disturbance or impact is then expressed as an ecological quality ratio (EQR) calculated as the observed state/the expected state. Defining the 'expected' state has been a major challenge for the past decade. In principle, palaeoecological studies provide a powerful means for doing this, albeit only in standing waters. Such an approach can be used to identify lakes whose present pH has changed little in recent times (cf. Bennion *et al*. 2004), which then serve as 'reference sites' (*sensu* Wallin *et al*. 2005) from which properties of biological quality elements in their near-pristine state can be measured for use as 'expected' values for EQR calculations.

Although metrics for assessing pH in running waters using diatoms have been developed (van Dam and Mertens 1995, Coring 1996, Kwandrans 2007, Andrén and Jarlman 2009) none are suitable as assessment systems for the WFD for two reasons: they do not address the issue of 'expected' values of metrics and they do not provide an ecological basis for setting quality status boundaries. The former is particularly significant as it is important that ecological status assessments can distinguish those water bodies which have been acidified by human causes from those which are naturally acid. An ecological rationale for setting boundaries is also important as the WFD provides, in Annex V (European Union 2000), ecological criteria by which ecological status should be assessed, and expects Member States to take action in those water bodies that achieve moderate status or less in order that they achieve good ecological status by 2015. The method described here is conceptually similar to that of Kelly *et al*. (2008) and will sit alongside this metric plus a suite of metrics based on macrophyte community composition to provide a suite of tools for the holistic assessment of the quality element 'macrophytes and phytobenthos' in the UK.

2 Methods

2.1 Dataset

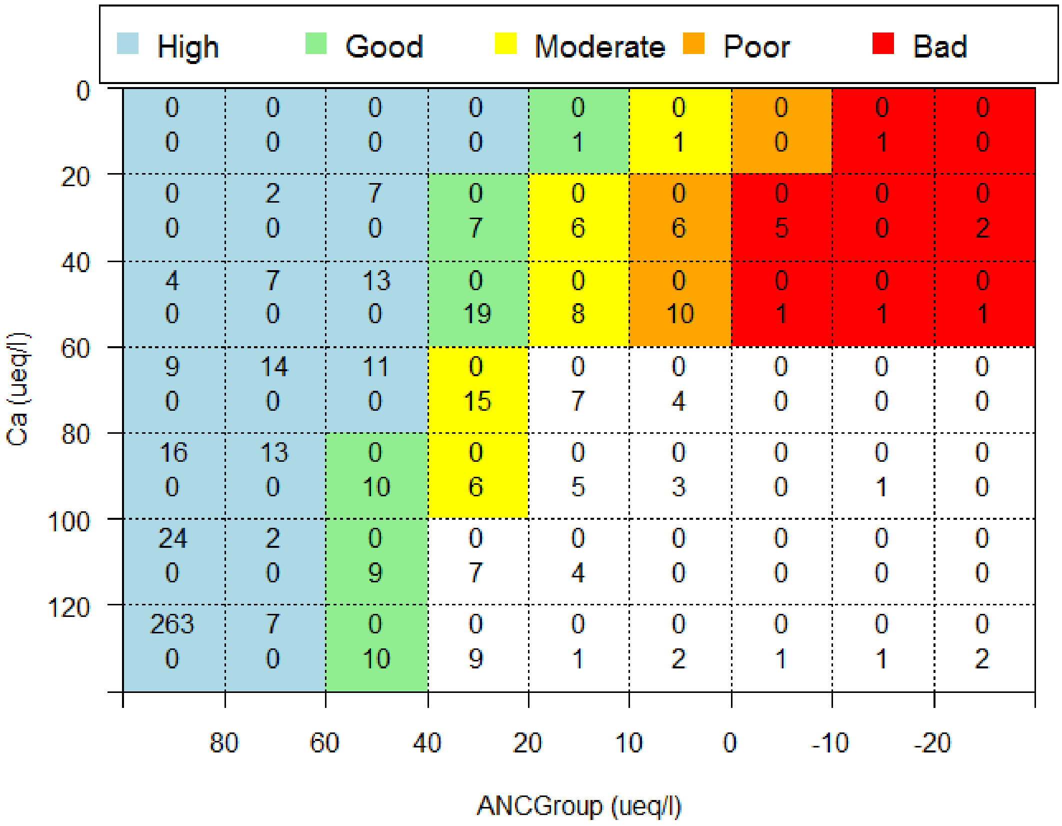
Data were derived from a number of previous projects: the Critical Loads of Acidity and Metals (CLAM) project, funded by Defra, consisting of 313 samples from the Acid Waters Monitoring Network, the Welsh Acid Waters Survey and data collected specifically for the CLAM project, and 245 samples from the ForWater project in the Republic of Ireland. These studies focused on those areas of the UK associated with hard rock geology, low alkalinity and, therefore, susceptibility to acidification. Sampling areas were principally in upland regions of south-west England, the Pennines, Wales, the Lake District, Scotland and Ireland, along with lowland samples from the New Forest (Hampshire) and Ashdown Forest (Sussex).

In the current study, five cobbles were collected from mid-stream and placed into a tray with a little stream-water, and the top surface of each was brushed with a clean toothbrush in order to remove the biofilm (Kelly *et al*. 1998, CEN 2003, 2004). The resulting suspension was collected in a plastic bottle, fixed with Lugol's iodine and stored prior to analysis. Samples were digested either in a saturated solution of potassium permanganate and concentrated hydrochloric acid (after Hendey 1974) or with hydrogen peroxide. Permanent slides were prepared using Naphrax (refractive index = 1.74) as a mountant. All nomenclature was adjusted to that used by Whitton *et al*. (1998), which follows conventions in Round *et al*. (1990) and Fourtanier and Kocoiolek (1999). All taxa were identified to the highest resolution possible (usually species or variety). Infraspecific taxa were merged for those species where a preliminary examination of taxon–environment scatterplots suggested that the response of infraspecific taxa was not distinguishable from that of the species. Detailed protocols for all methods are available at http://craticula.ncl.ac.uk/dares/methods.htm.

2.2 Definition of reference conditions

The unimpacted condition is defined here as a water body in which base cations are in balance with strong acid anions. This is in contrast with the situation that prevails in impacted water bodies, where there is a surfeit of acid anions relative to base cations, implying an elevated concentration of acid cations (H^+ and Al^{3+}). In practical terms, there should normally be a balance between base cations and 'acid neutralising capacity' (ANC) consisting of contributions from the bicarbonate–carbonate buffering system and dissolved organic carbon. As ANC falls as acidification proceeds (i.e. the ratio of acid anions to base cations increases), this alone is not useful for defining the unimpacted condition; however, there should be a relationship between ANC and base cation concentrations such that sites where these are in balance can be deemed to be 'unimpacted'. Selection of such sites was based on the 'damage matrix' of Monteith and Simpson (2007: Table 2.1), which uses expert judgement to assign likely ecological status on the basis of ANC relative to calcium (Ca). For this study, sites defined as 'high status' have high ANC relative to Ca and have been used as reference sites.

Table 2.1 Damage matrix of Monteith and Simpson (2007) based on understanding of relationships between ANC and Ca concentrations and evidence from palaeoecological and hydrochemical models of acidification, and contemporary relationships with macroinvertebrate assemblage characteristics. The different colours represent expert judgement of likely ecological status with respect to damage from acidification. Numbers in the cells indicate numbers of reference (upper) and non-reference (lower) samples in each ANC/Ca class



The approach used here differs from that recommended to define reference conditions for other pressures, where sites are screened using land-use data (Wallin *et al*. 2005, Stoddard *et al*. 2006) that would not be suitable for this type of pressure. This screening process yielded 369 potential reference samples, although 263 of these were associated with the highest Ca class (>120 μeq l^{-1}).

2.3 Derivation of metric

The method used was broadly similar to that used to develop the DARLEQ model (Diatom Assessment of River and Lake Ecological Quality; Kelly *et al*. 2008), except that the Trophic Diatom Index (TDI) was replaced by a new 'Diatom Acidification Metric' (DAM), derived by computing the pH optimum or preference value for each taxon using Gaussian logistic regression (ter Braak and Looman 1986). Taxa were then assigned to one of five pH groups on the basis of these optima as follows: 1 = pH <5.0; 2 = pH 5.0–5.6; 3 = pH 5.6–7.0; 4 = pH 7.0–8.0; 5 = pH >8. The ranges of the five pH classes approximately match the pH classes defined by Hustedt (1938–1939; see van Dam *et al*. 1994). Furthermore, of 129 taxa recorded in this study, 97 were recognised by

Hustedt (1938–1939) and, of these, 43% had identical classes in the two systems while 86% differed by no more than one class. The taxon scores were then used to calculate an acidification score for each sample by weighted averaging, with the final sample scores rescaled to run from 0 (low pH) to 100 (high pH). The relationship between the DAM and pH and DAM and ANC is shown in Figure 2.1. DAM shows a higher correlation to pH (r = 0.81) than ANC (r = 0.65), indicating that DAM is a good indicator of acidity, rather than acidification.

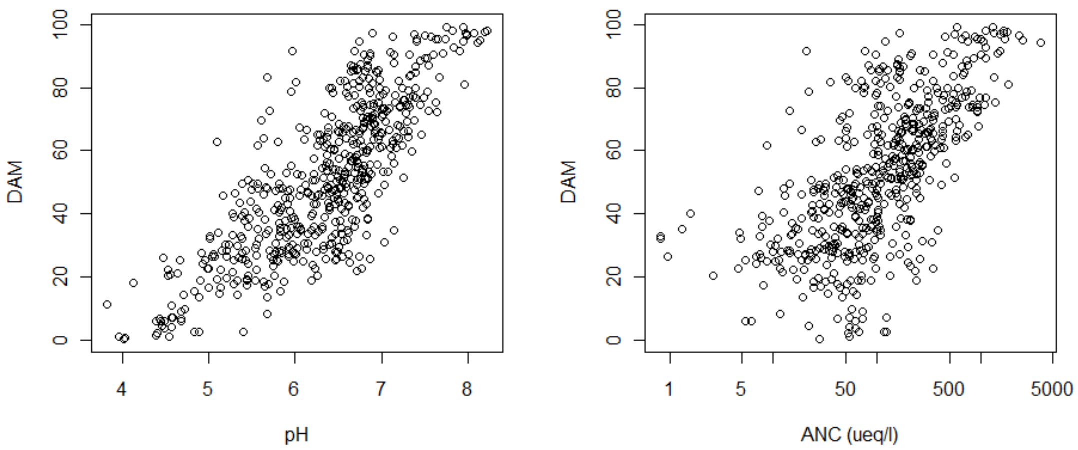


Figure 2.1 Relationship between DAM and pH (left) and ANC (right)

3 Results

3.1 Description of dataset and preliminary ordinations

In total, 365 species or varieties of diatom with more than two occurrences were identified. The most frequently identified taxa were *Eunotia exigua* (65% of samples), *Tabellaria flocculosa* (60%), *Fragilaria capucina* (54%) *Achnanthidium minutissimum* type (52%), and *E. incisa* (44%); 45 taxa were found in fewer than ten samples. The maximum relative abundance of any taxon in a sample was 97% for *Eunotia exigua* followed by 96% for *E. intermedia*. A total of 57 taxa occurred at 20% or more in at least one sample (Figure 3.1).

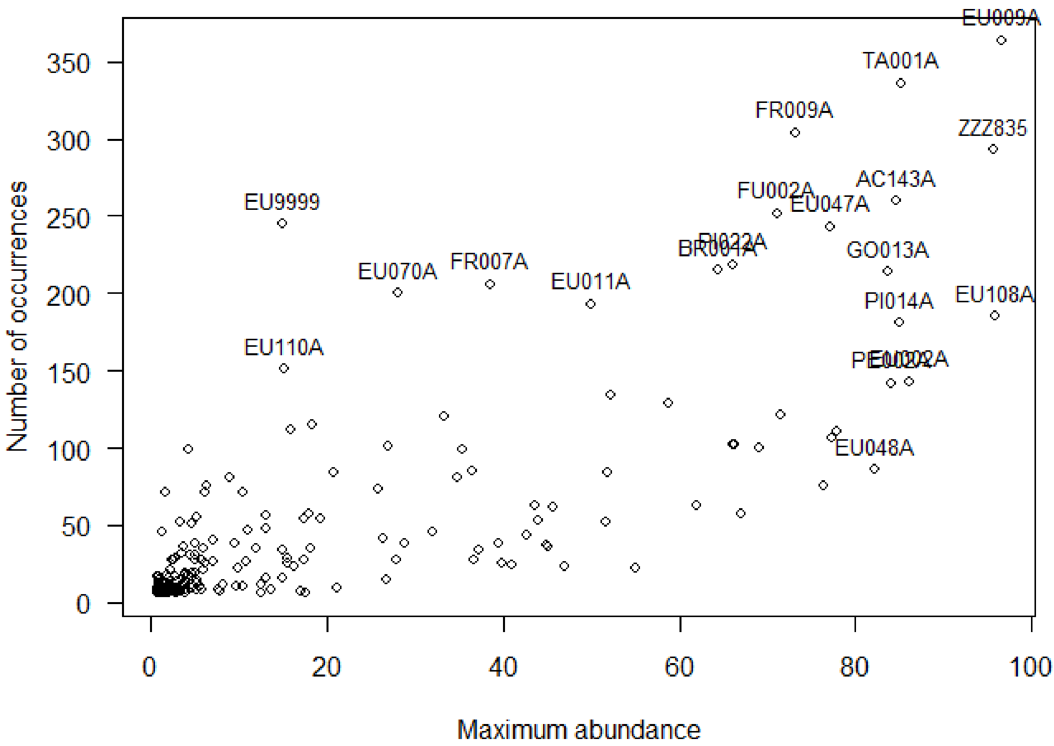


Figure 3.1 Relationship between number of occurrences and maximum abundance (as a percentage) for all taxa with more than two occurrences in the full dataset. See the Appendix for key to taxon names

Figure 3.2 shows a canonical correspondence analysis (CCA) biplot of the full dataset. Axis 1 accounts for 4% of the variation in the diatom data and is related to the acidity gradient. Axis 2 accounts for an additional 2.6% of variation and is primarily related to total organic carbon (TOC). Although the explained variation is small these values are typical for large, species-rich datasets with many zeros in the data matrix, and both axes are highly significant (p = 0.001, Monte Carlo permutation test, 999 permutations).

Circumneutral taxa such as *Cocconeis placentula* and *Amphora pediculus* typically had positive scores on axis 1, while acid-tolerant taxa had low or slightly negative scores and were arranged along the second axis. This may reflect, in part, a gradient from taxa typical of habitats rich in humic materials (e.g. *Frustulia saxonica*), which have low scores on axis 2, to those typical of clearwater acid habitats (e.g. *Peronia fibula*), which tend to have higher scores.

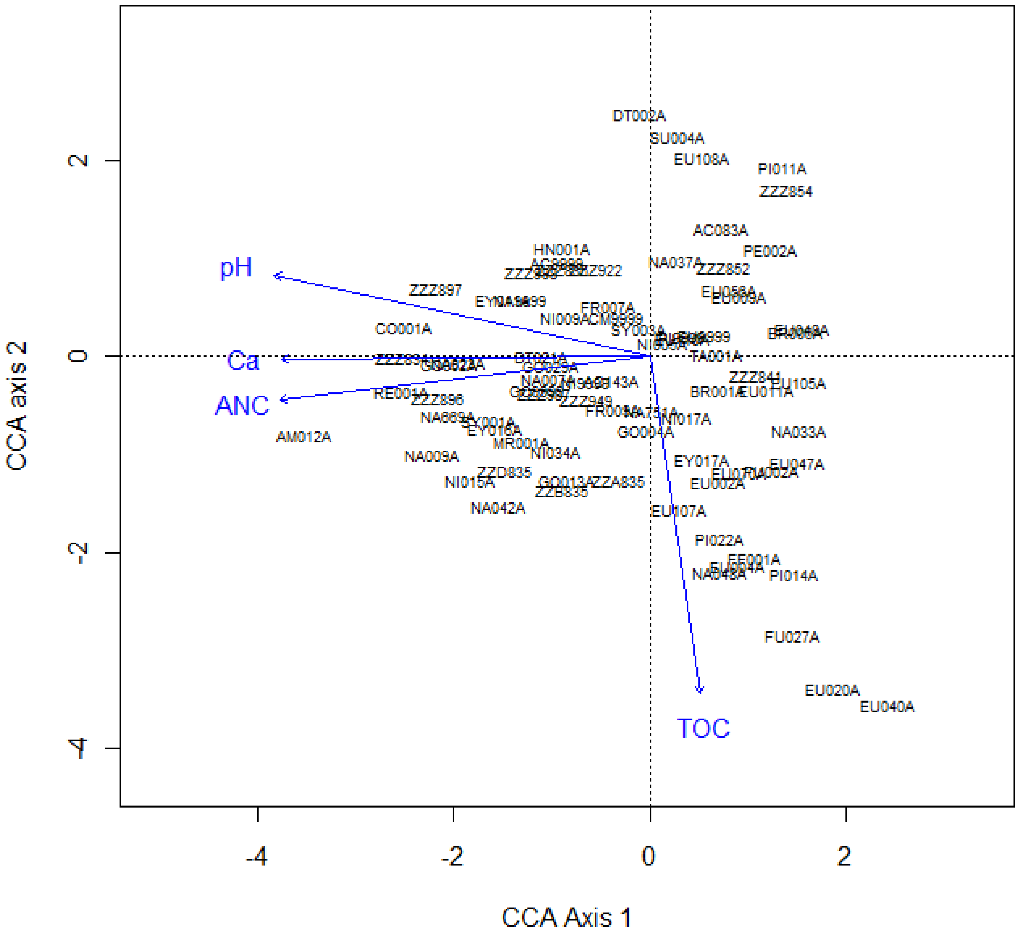


Figure 3.2 Canonical correspondence analysis biplot showing taxa and environmental variables. Only taxa with maximum relative abundance greater than 30% are included. Taxon codes and names are listed in the Appendix

3.2 Properties of reference sites

The composition of samples at reference varied from domination by circumneutral taxa such as *Achnanthidium minutissimum* when Ca concentrations were >100 μeq l^{-1} to domination by *Eunotia* spp. and *Tabellaria flocculosa* in the most soft-water sites (Figure 3.3). Alongside *A. minutissimum*, *Fragilaria capucina* var. *gracilis* is also abundant. *Brachysira vitrea* and *Gomphonema parvulum* (and varieties) were also common. *Achnanthes oblongella* is almost absent when Ca >300 μeq l^{-1} but forms up to about 40% of the total when Ca = 100 μeq l^{-1}, before declining again and becoming rare in the softest water (<50 μeq l^{-1}). As Ca concentrations decline, so the proportion of *Eunotia* spp. (especially *E. exigua, E. intermedia* and *E. incisa*) increase, along with *Peronia fibulata, Pinnularia subcapitata* and *P. appendiculata. Navicula sensu stricto* and *Nitzschia* spp. are both relatively rare in these waters.

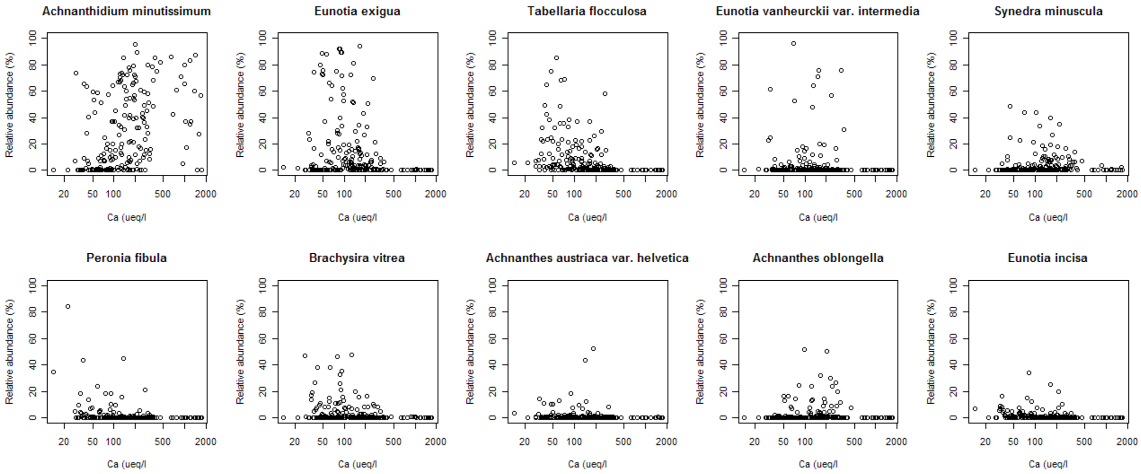


Figure 3.3 Relative abundance of the ten most abundant taxa in reference samples

3.3 Derivation of ‘expected’ values of DAM and calculation of EQRs

Calculation of an EQR for a sample requires an estimate of the ‘expected’ value of DAM for the site under reference conditions. To be useful this estimate has to be derived from routinely measured data, or from variables that the agencies do not routinely measure but that could be added relatively easily. The ‘expected’ values should also be estimated using variables not related to the underlying pressure of interest, since the ‘expected’ or reference value is an intrinsic characteristic of the site that is independent of the pressure gradient. Given these criteria we tested models using calcium and TOC as predictors. Calcium is a simple measure of the buffering capacity of a site and, apart from a small effect described above, is invariant with acidification. Similarly, TOC concentration is an intrinsic feature of the site that is primarily controlled by catchment characteristics. In oligotrophic waters it primarily reflects the concentration of humic acids, which represents both a direct source of weak acidity and also a buffer against the toxic effects of dissolved aluminium (Al) at low pH. Consequently, it is included as a potential predictor of ‘expected’ values for DAM.

Models were developed using multiple regression and were fitted hierarchically, testing the significance of each regression term against the previous model. Table 3.1 summarises the fitting procedure and model performance. All terms were significant except TOC^2. The model with TOC and Ca shows significant improvement over the single term model, and therefore this was adopted. The final model is:

$$\text{Expected DAM} = -5.5 + 33 * \log_{10}(\text{Ca}) - 1.9\ \text{TOC} \quad \text{(Equation 3.1)}$$

Table 3.1 Summary statistics for the regression models for predicting DAM. Adj-R^2 is squared correlation between observed and predicted values, adjusted for the number of predictors. RMSE is the root mean squared error of prediction of DAM

Explanatory variables	Adj-R^2	RMSE
Ca	0.38	17.2
Ca + TOC	0.54	14.7
Ca + TOC +TOC^2	0.54	14.5

Figure 3.4 shows the relationship between observed and predicted DAM for the reference samples. There is a slight tendency to underestimate DAM at high values and overestimate at low. EQRs were then calculated using the relationship EQR = O/E, where O is the observed and E is the expected values, predicted using the regression model defined above.

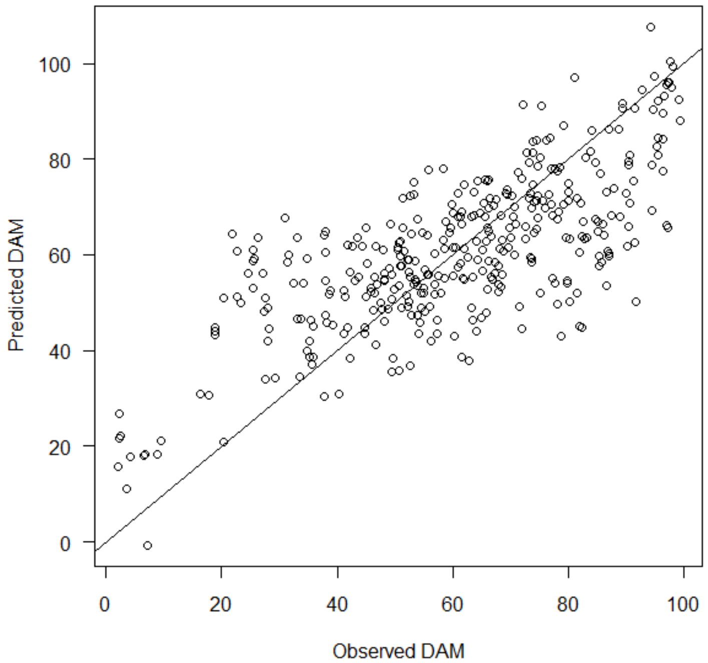


Figure 3.4 Relationship between observed and predicted DAM for reference samples. The axes are scaled in DAM units which range from 0 to 100

3.4 Derivation of status class boundaries

Figure 3.5 shows the distribution of EQRs in the reference dataset. The majority fall between 0.8 and 1.2 but there are a few reference samples with EQRs less than 0.2, suggesting that the reference dataset contains a number of samples from impacted sites. A cautionary approach was therefore adopted in setting the high/good status boundary. This was set at the 25th percentile of the reference EQRs to allow for the fact that some samples may not be at reference. EQRs greater than 1.0 were set to 1.0 in subsequent calculations.

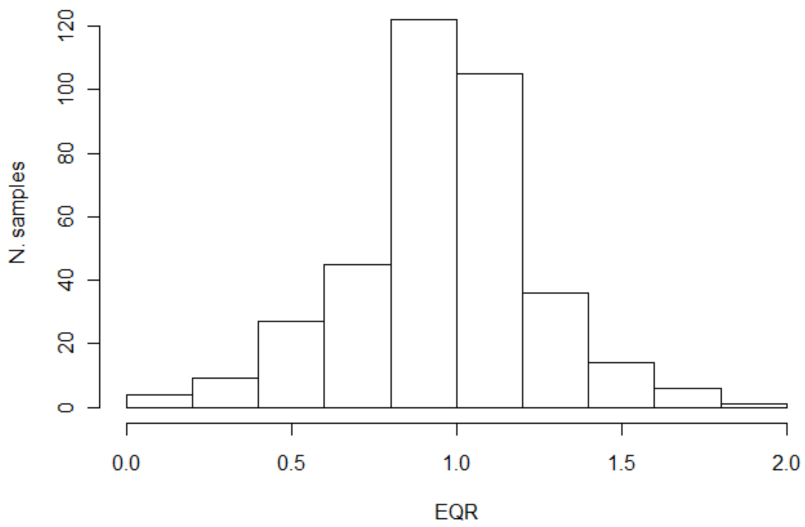


Figure 3.5 Distribution of EQRs in the reference dataset

In order to define the good/moderate status class boundary we used the predicted regression model defined above to calculate the ‘expected’ values and EQRs for the full dataset (i.e. both reference and non-reference samples). When EQR is high, samples are dominated by acid-sensitive taxa (defined as taxon groups 1–2) while as EQR declined (representing an increase in acidification pressure) so the proportion of acid-tolerant taxa increased (Figure 3.6).

The point at which the ‘sensitive’ taxa cross the ‘tolerant’ taxa (the ‘cross-over’) represents the point at which assemblages typical of reference conditions become subordinate to assemblages indicative of acidification and this was chosen as a suitable location for the good/moderate boundary (Figure 3.7). The moderate/poor and poor/bad status class boundaries are set at equal points below the good/moderate boundary. The resulting class boundaries are listed in Table 3.2.

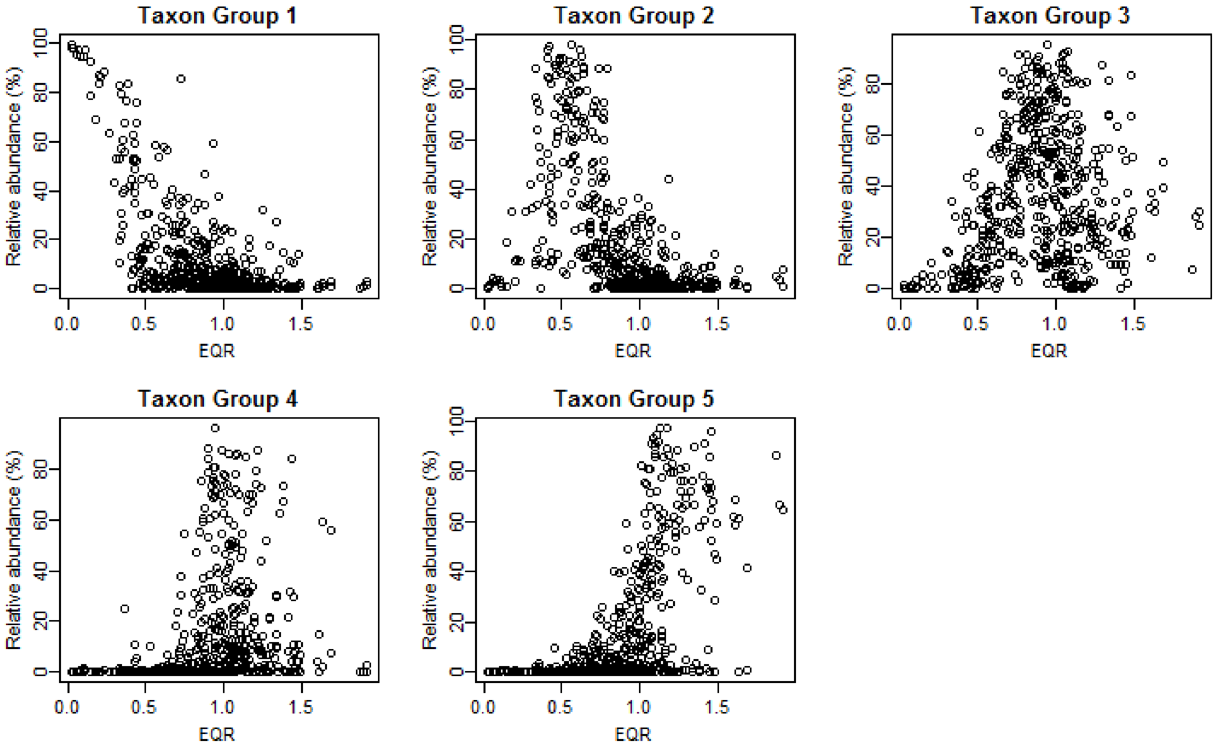


Figure 3.6 Scatterplots showing the distribution of taxon groups 1–5 with EQR

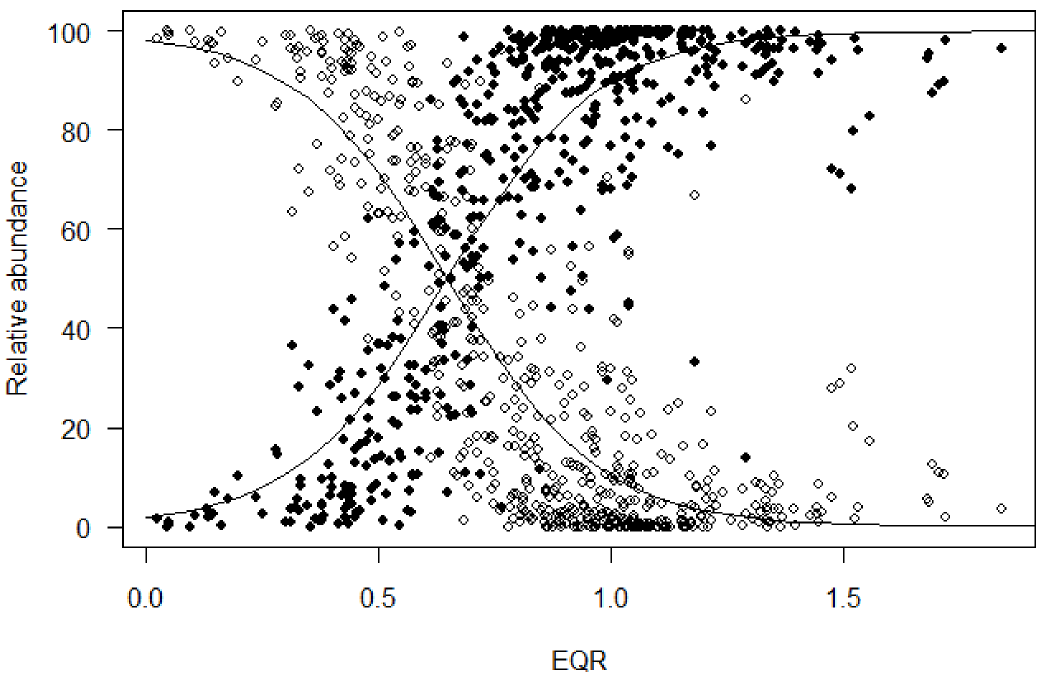


Figure 3.7 Relationship between the relative abundance of acid-sensitive (open) and acid-tolerant (filled) taxon groups and EQR. Lines show fitted logistic regression models used to identify the cross-over point

Table 3.2 Status class boundaries for use with the DAM tool

Explanatory variables	H/G	G/M	M/P	P/B
EQR	0.81	0.65	0.44	0.22

3.5 Validation of the DAM tool

A validation exercise using a test dataset of samples from the UK Acid Waters Monitoring Network (UKAWMN) was carried out. This dataset contains annual epilithic diatom samples from 11 stream sites beginning in 1988, and spans a range of sites from sensitive unimpacted streams in north-west Scotland to chronically acidified sites in southern England. Full associated chemical and other biological data are also available and the sites have been the subject of several detailed investigations into their chemical and biological status and trajectories (Kernan *et al*. 2010, http://awmn.defra.gov.uk/). Three years of data from 1990 to 1992 were selected for use (Table 3.3).

The diatom assessment agreed well with status derived from long-term chemical and biological monitoring. The tool discriminates between high, moderate and poor sites, but fails to identify mildly acidified sites subject to acid episodes at high flow. This is likely to be because of the time of sampling: the validation diatom samples were collected over summer during predominantly low flow conditions when sites are buffered by input of cations from baseflow. This sampling period misses episodic winter/early spring/late autumn low pH and low alkalinity high flow events. This effect is particularly apparent in samples from the River Etherow, where two samples capture higher pH conditions characterised by *Achnanthidium minutissimum* assemblages and only one sample, collected in 1991 and characterised by the acid-tolerant taxon *Eunotia exigua*, reflects the chronically acidified nature of this site.

Table 3.3 DAM-derived quality classes for the UKAWMN validation sites and summary of status based on long-term chemical and biological monitoring

Site	Status 1990	1991	1992	Status derived from chemical and other biological monitoring
Allt a'Mharcaidh (Cairngorms)	High	High	High	Well-buffered stream in area of low deposition. Occasional episodic pH depression but alkalinity >0
Allt na Coire nan Con (NW Scotland)	High	High	High	Mildly acidic site with occasional acid episodes. Strong seasonality in chemistry
Dargall Lane (Galloway)	Moderate	Poor	Moderate	Mildly acidic site suffering frequent acid episodes that reduce pH to <5 and alkalinity to <0
River Etherow (Pennines)	High	Poor	High	Impacted site with high SO_4 and NO_x, but highly episodic and well buffered by catchment cations at low flow
Old Lodge (SE England)	Poor	Poor	Poor	Severely acidified with mean pH <5 and almost continuous negative alkalinity
Narrator Brook (Dartmoor)	High	High	High	Mildly acidic with occasional acid episodes that cause alkalinity to fall below zero
Afon Hafren (Plynlimon)	Moderate	Moderate	Moderate	Moderately acidic site with frequent acid episodes and occasional negative alkalinity
Afon Gwy (Plynlimon)		Moderate	Moderate	Moderately acidic site with frequent acid episodes and occasional negative alkalinity
Beagh's Burn (Co. Antrim)	Moderate	High	High	Mildly acidic, relatively unimpacted site with occasional acid episodes
Bencrom River (Mourne Mtns)	Moderate	Poor	Poor	Severely acidified site with frequent low pH (<5) and long periods of negative alkalinity
Conyglen Burn (Sperrin Hills)	High	High	Good	Relatively unimpacted but subject to occasional mild acid episodes

4 Discussion

The importance of pH in controlling the distribution and abundance of diatoms in freshwater systems has long been recognised (e.g. Hustedt 1938–1939). The strength of this relationship has been exploited in palaeolimnology, where it has been quantified in transfer functions (e.g. Birks *et al*. 1990) and used to reconstruct past changes in lake-water pH from subfossil diatom assemblages preserved in lake sediments (Battarbee *et al*. 2010). Similar strong relationships between diatom assemblage composition and mean pH have also been observed in flowing waters (e.g. Andrén and Jariman 2008, Stevenson *et al*. 2010).

pH is a primary factor controlling assemblage composition in the DARLEQ dataset and this relationship has been encapsulated in the new Diatom Acidification Metric (DAM). The correlations between the new DAM and measured mean stream-water pH were high, with values of $r = 0.81$ and $r = 0.77$ for the full and reference datasets, respectively. These results, and those from other studies, indicate that diatoms provide a tool for the bioassessment of stream-water acidity. However, the challenge in developing a metric for assessing ecological status in soft-waters is to differentiate between those water bodies that are naturally acid and those that have been acidified due to human impact. Palaeolimnology has played an important role in establishing pre-impact conditions in lakes (Battarbee *et al*. 1999) but no equivalent methods have yet been developed for running waters. Evidence presented here (Figure 3.3, Table 2.1) shows large changes in the diatom flora along a gradient of soft-water sites presumed to be free from anthropogenic impacts, leading to changes in the 'expected' value of DAM.

Variables used to predict 'expected' values of metrics need to be independent of the pressure under consideration, ruling out the use of alkalinity, as used for evaluations of nutrient impacts (Kelly *et al*. 2008). Calcium, derived largely from catchment geology, is also a reliable predictor of the expected flora; however, it, too, is not wholly immune from the pressure. Keller (2009) presents data showing a decline in Ca concentration in Clearwater Lake, near Sudbury, Ontario, as pH increased during recovery from acidification. This reflects both reduced chemical weathering and increased availability of cation binding sites as H^+ concentrations decline. The relationship between current and pre-industrial base cations has been modelled in the Steady State Water Chemistry (SSWC) model, Henriksen *et al*. (1992). Unfortunately, the majority of sites in the DARLEQ database lack the sulphate and nitrate data necessary for detailed modelling but studies elsewhere suggest that the increase in base cations as a result of acidification is less than 20% for the majority of sites, and less than 15% for sites with Ca < 50 $\mu eq\ l^{-1}$.

This work has shown evidence of natural long-term changes along a Ca gradient, even in the absence of acidification. Problems will also arise if the present system is used to evaluate changes when liming is used as a remediation technique. Liming will also have additional ancillary effects that may influence the diatom assemblage (e.g. phosphorus sequestration: see Anderson *et al*. 1997). Adding lime will, by raising the Ca concentration, alter the 'expected' value. However, the gradual removal of the lime from the catchment may lead to a fall in Ca and re-establishment of an acid flora (e.g. Bellemakers and van Dam 1992). Monitoring would consequently take place against a fluctuating baseline, while the remediation itself may mean that the true 'expected' flora will never be regained.

The bicarbonate system is the predominant buffer for most non-acid UK waters although TOC, representing the input of weak organic acids, becomes important at low

Ca (Schneider 2010). There is evidence for this in the regression model for the 'expected' DAM, where TOC has a negative coefficient (Equation 3.1). Calcium and TOC, as predictors of the expected flora under reference (pre-industrial) conditions, explain approximately half the variation in DAM. This is a reasonable proportion given the uncertainties in both the diatom and hydrochemistry data and the relative simplicity of the predictive model. A similar model developed for assessing nutrient impacts explained only 33% of the expected index (Kelly *et al*., 2008). Like Ca, TOC may also exhibit long-term trends; however, as TOC explains a small part of the total variation in DAM at reference, the influence of this is likely to be minor. The model for 'expected' values in DAM was generated from a relatively large dataset of reference samples, although most of these samples fell in the range pH 6.5–7.0. The reference dataset contained few sites with pH <6, which accounts for some of the bias in the regression model (Figure 3.4), and the over-prediction of DAM in more acid waters. Future work should prioritise samples from low pH reference systems.

The model was validated using time-series data from a number of well-studied catchments. In most cases the predicted status class agreed well with the status derived from chemical and invertebrate analyses. Where there was disagreement this could be attributed to the episodic nature of the sites. Diatoms can respond to changes in water chemistry in a matter of days (e.g. Hirst *et al*. 2004) and recent work has shown that diatoms exhibit different responses to chronic and episodic acidification (e.g. Passy 2006). Unfortunately, detailed time-series chemistry is not available for the DARLEQ samples so it was not possible to explore the response of diatom community structure to episodic versus mean chemistry in this study. However, given the rapid temporal response of diatoms to changes in chemistry it is clearly important to sample at the appropriate time of year to encompass any episodes of low pH and ANC during periods of high flow. If appropriate sampling can be achieved then the new DAM provides a relatively simple but effective way of assessing the ecological status of UK soft-waters.

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Appendix: List of codes and taxon names shown in figures

Code	Name	DAM score
AC083A	*Achnanthes laevis*	2
AC143A	*Achnanthes oblongella*	3
AC182A	*Achnanthes rosenstockii*	3
AM012A	*Amphora pediculus*	5
BR001A	*Brachysira vitrea*	3
BR006A	*Brachysira brebissonii* fo. *brebissonii*	2
CM004A	*Cymbella microcephala* fo. *microcephala*	3
CM022A	*Cymbella affinis*	5
CO001A	*Cocconeis placentula*	5
DE001A	*Denticula tenuis*	4
DT002A	*Diatoma hyemale* var. *hyemale*	3
DT021A	*Diatoma mesodon*	4
EU002A	*Eunotia pectinalis*	2
EU003A	*Eunotia praerupta*	1
EU004A	*Eunotia tenella*	1
EU009A	*Eunotia exigua*	2
EU011A	*Eunotia rhomboidea*	1
EU013A	*Eunotia arcus*	1
EU015A	*Eunotia denticulata* var. *denticulata*	2
EU020A	*Eunotia meisteri*	1
EU026A	*Eunotia praerupta-nana*	3
EU040A	*Eunotia paludosa*	1
EU040B	*Eunotia paludosa* var. *trinacria*	1
EU047A	*Eunotia incisa*	1
EU048A	*Eunotia naegelii*	2
EU049D	*Eunotia curvata* var. *attenuata*	3
EU053A	*Eunotia tridentula*	3
EU070A	*Eunotia bilunaris*	1
EU105A	*Eunotia subarcuatoides*	2
EU107A	*Eunotia implicata*	3
EU108A	*Eunotia intermedia*	3
EU110A	*Eunotia minor*	3
EU114A	*Eunotia muscicola*	3
EU9999	*Eunotia* sp.	2
EY011A	*Encyonema minutum*	4
EY016A	*Encyonema silesiacum*	4
EY017A	*Encyonema gracile*	3
FF001A	*Fragilariforma virescens*	1
FR007A	*Fragilaria vaucheriae*	3
FR009A	*Fragilaria capucina*	3
FR026A	*Fragilaria bidens*	4
FU002A	*Frustulia rhomboides*	1
FU027A	*Frustulia saxonica*	1
GO001A	*Gomphonema olivaceum*	4
GO003A	*Gomphonema angustatum*	3
GO004A	*Gomphonema gracile*	3

GO013A	*Gomphonema parvulum*	4
GO029A	*Gomphonema clavatum*	4
GO052A	*Gomphonema olivaceoides*	4
HN001A	*Hannaea arcus*	4
MR001A	*Meridion circulare*	4
NA009A	*Navicula lanceolata*	4
NA023A	*Navicula gregaria*	4
NA042A	*Navicula minima*	4
NA138A	*Navicula pelliculosa*	4
NA352A	*Navicula evanida*	4
NA415A	*Navicula harderi*	3
NA669A	*Navicula suchlandtii*	4
NA751A	*Navicula cryptotenella*	3
NI002A	*Nitzschia fonticola*	5
NI009A	*Nitzschia palea*	4
NI015A	*Nitzschia dissipata*	4
NI025A	*Nitzschia recta*	3
NI033A	*Nitzschia paleacea*	4
PE002A	*Peronia fibula*	2
PI014A	*Pinnularia appendiculata*	1
PI022A	*Pinnularia subcapitata*	1
RE001A	*Reimeria sinuata*	4
SA012A	*Stauroneis kriegeri*	3
SS002A	*Staurosirella pinnata*	5
SU004A	*Surirella biseriata* var. *biseriata*	3
SY001A	*Synedra ulna*	4
TA001A	*Tabellaria flocculosa*	3
TA004A	*Tabellaria quadriseptata*	1
TA006A	*Tabellaria ventricosa*	2
ZZZ834	*Gomphonema angustum/pumilum* type	5
ZZZ835	*Achnanthidium minutissimum* type	5
ZZZ841	*Fragilariforma exigua*	2
ZZZ852	*Psammothidium helveticum*	3
ZZZ853	Temporary sp145	3
ZZZ854	*Psammothidium marginulatum*	3
ZZZ896	*Planothidium frequentissimum*	4
ZZZ897	*Planothidium lanceolatum*	5
ZZZ911	*Achnanthidium subatomus*	5
ZZZ922	*Planothidium* sp.	3
ZZZ949	*Psammothidium subatomoides*	3

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