Final Report **Project WFD60**

Macroinvertebrate classification diagnostic tool development

August 2007

Dissemination status

Unrestricted

Use of this report

The development of UK-wide classification methods and environmental standards that aim to meet the requirements of the Water Framework Directive (WFD) is being sponsored by UK Technical Advisory Group (UKTAG) for WFD on behalf its member and partners.

This technical document has been developed through a collaborative project, managed and facilitated by SNIFFER and has involved the members and partners of UKTAG. It provides background information to support the ongoing development of the standards and classification methods.

Whilst this document is considered to represent the best available scientific information and expert opinion available at the stage of completion of the report, it does not necessarily represent the final or policy positions of UKTAG or any of its partner agencies.

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EXECUTIVE SUMMARY

WFD60: Macroinvertebrate diagnostic tool development (August, 2007)

Project funders/partners: SNIFFER

Background to research

This project (WFD60) forms part of the UK Strategy for the implementation of the EC Water Framework Directive (WFD: European Union, 2000). Within its broad remit the WFD requires the development of ecological classification tools for the purpose of determining ecological status, with reference to specific environmental pressures. The WFD requires that these tools should assign lakes to one of five categories, (High, Good, Moderate, Poor, Bad) to indicate conditions relative to what is considered to be "good status". This report focuses on the development of a tool with which to determine the extent of the pressure of acidification on lake macroinvertebrate communities.

Objectives of research

The primary objective is the development of a method and tool with which to assess the pressure of acidification (a major threat to the ecology of acid-sensitive fresh waters, particularly in the UK uplands) on the benthic macroinvertebrate assemblage of lakes.

Key findings and recommendations

Tool development under WFD60 was severely delayed due to problems obtaining sufficient high quality biological and chemical data. The dataset used to support this phase is still less than satisfactory, comprising data for only 105 sites and representing a subset only of the chemical variables that would have been useful for explanatory data analysis. Due to the paucity of acid anion data from one source and dual endpoint (or Gran) alkalinity from another, the final physico-chemical dataset was built using one of two commonly used expressions of acid neutralising capacity (ANC) and a few associated determinands.

Our assessment of the literature regarding macroinvertebrate-acidification inference techniques concluded that none were appropriate for this assignment. In most cases macroinvertebrate communities have been used to infer pH, but pH per se carries little information on acid sensitivity or the likelihood that a site has acidified.

We show, through an investigation of the output of the Steady State Water Chemistry (SSWC) Model and palaeoecological diatom-pH reconstructions, how ANC can be used as an indicator of damage, in terms of modelled ANC change, diatom-inferred pH change and the mobilisation of labile inorganic aluminium (Al_{lab}) concentration.

Furthermore, we show that prediction of the likelihood and level of acidification can be refined by using ANC in conjunction with calcium concentration.

Assessment of chemical data from the UK Acid Waters Monitoring Network demonstrates that Allab concentration, possibly the most important agent of damage associated with acidification, will rarely if ever reach biologically toxic concentrations in sites with an ANC above 40 µeq l^{-1}. Conversely, sites which currently have a negative ANC are highly likely to exhibit biologically toxic Al_{lab} concentrations.

We show that ANC and Al_{lab} explain as much variance in a small high quality macroinvertebrate dataset as pH and propose that macroinvertebrate community structure may carry sufficient information for the level of physico-chemical damage to be inferred through its relationship with ANC and calcium concentration.

In the expanded dataset, representing 105 lakes, we again show that ANC is strongly related to the principal axis of macroinvertebrate species variation between sites.

We show that certain attributes of macroinvertebrate community structure pertinent to normative definitions also vary along an ANC gradient. In particular, a crude measure of macroinvertebrate species richness, as inferred by the total number of species identifiable to species level, is tightly related to ANC. This is consistent with observations in the literature that macroinvertebrate diversity may be reduced by anthropogenic acidification but not by natural acidity (i.e. at sites where pH is depressed by organic acids only). Several individual species show sharply truncated distributions on Al_{lab} gradients and species often ceased to be present in waters with mean annual Al_{lab} concentrations over 10 µg l^{-1}.

We created a “damage matrix” to provide an *a priori* physico-chemical classification of all sites in the WFD60 database by ANC and calcium concentration into WFD compliant classes, i.e. HIGH, GOOD, MODERATE, POOR, BAD. Owing to the sparsity of the data we then condensed these classes into three representing HIGH-GOOD, MODERATE and POOR-BAD.

We used a classification tree approach to predict the *a priori* defined class of each site using its macroinvertebrate assemblage. Classification trees are a powerful yet simple way of predicting classes from a set of predictor variables (in this case, macroinvertebrate species and broader macroinvertebrate groups).

After using a large range of biological input variables, including data at species level (i.e. the proportions of individual taxa) we found that summary data only, in the form of minimum species richness (MSR) of the full assemblage, the minimum number of species in certain biological groups, and the proportion of individuals represented by certain groups, was necessary to maximise the successful classification rate. The final tree classification used these variables only.

We found that a simple rule, i.e. MSR >or<12.5, provided the most powerful criterion for distinguishing between damage classes at the primary level. Further splits were based on the number of non-leptophebid (i.e. mostly acid-sensitive) Mayfly taxa, the presence/absence of bivalves, the proportion of Ephemeropteran, Plecopteran and Trichopteran individuals in the entire assemblage, and the minimum number of stonefly taxa. The apparent misclassification rate of this tree was 18.3%. We determined that the tree should be able to correctly assign class status to random independent samples between 77 – 78% of the time.

This simple approach was able to distinguish between acidified and naturally acid (i.e. high DOC, low sulphur deposition) lakes that tend to support relatively large numbers of taxa. Apparently more complex, species-based, models such as the Acid Water Indicator Community model (AWIC) are perhaps better tuned to predict pH but have limited value in this sense.

While the divisions on this tree form our current “best” model, we have major reservations with respect to the total number of sites in the dataset and the distribution of sites at the acidified end of the gradient. The model as it stands is clearly not fit for purpose but would benefit greatly from the addition of 30-40 more sites in an acidified condition.

While this is a categoric approach to classification, class predictions could be converted to EQR-compatible site scores to meet WFD requirements. There are a number of methods to

achieve this, but the most robust would use a method known as “bagging” to determine the probability of membership of each site in the most likely class and neighbouring classes, to provide a sliding score. The proposed increased number of sites would be essential for this technique to be used effectively.

We tested the tool qualitatively on 51 sites for which chemical data were not adequate to be included in the original training set. Generally the classification of sites was highly consistent with geographical location although a few sites were clearly misclassified.

Current model weaknesses are likely to be principally due to the paucity of sites for which data are available at the acid and acidified end of the physico-chemical gradient. The imbalance of sites in the training set also prevents us from deriving predictions of the probability of correct classification using a “tree bagging” technique.

We recommend that biological and physico-chemical data are gathered for a further 30-40 acidified sites before any attempt is made to refine the existing model.

Before implementation, we recommend the tool is tested on 1) time series data, to allow an assessment of temporal variability of output, and 2) sites for which detailed multi-proxy biological records are available, so that the macro-invertebrate inferred damage class can be related to wider-ecosystem indications of damage by acidification.

Key words: Water Framework Directive, Lakes, Acidification, Littoral Macroinvertebrates, Classification, Classification Trees, Acid Neutralising Capacity, Aluminium, pH.

TABLE OF CONTENTS

List of Tables

List of Figures

APPENDICES

1. INTRODUCTION

This project (WFD60) forms part of the UK Strategy for the implementation of the EC Water Framework Directive (WFD: European Union, 2000). Within its broad remit the WFD requires the development of ecological classification tools for the purpose of determining ecological status, with reference to specific environmental pressures. Our primary objective is the development of a method and tool with which to assess the pressure of acidification (a major threat to the ecology of acid-sensitive fresh waters - particularly in the UK uplands) on the benthic macroinvertebrate assemblage of lakes.

The WFD requires that the tool should assign lakes to one of five categories, (High, Good, Moderate, Poor, Bad) to indicate conditions relative to what is considered to be “good status”. Inherent errors are to be defined and quality assurance provided. Ultimately, the tool will be provided in the form of simple software that will allow the funding agencies to test further datasets.

In this report we review WFD lake classification requirements and our understanding of how these may apply to the issue of macroinvertebrates and acidification. We describe the physico-chemical and biological database compiled under the project and demonstrate links between acidification pressure metrics and macroinvertebrate normative definition compatible characteristics of lakes. We briefly consider the philosophy behind the ongoing development of other classification tools within the UK before presenting an alternative approach, based primarily on the concept of classification trees, which is used in WFD60 to underpin the tool.

1.1 The Water Framework Directive and Macroinvertebrates

The Water Framework Directive (WFD) requires EU Member States to monitor the ecological status of water bodies with the aim of achieving ‘good ecological status’ for all water bodies by 2015. The Directive provides normative definitions for biological status classification (Annex V) and these are summarised for macroinvertebrates in Table 1.1. Waters deemed less than moderate are classed either poor (major alterations to ecological quality) or bad (severe alterations to ecological quality).

Table 1.1 WFD Normative definitions for biological elements (macroinvertebrates) relating to High Good and Moderate status.

Feature	High status	Good status	Moderate status
Taxonomic composition and abundance.	Corresponds totally or nearly totally to undisturbed conditions.	Slight changes from the type-specific communities.	Differ moderately from the type-specific communities. Major taxonomic groups of the type-specific community are absent.
Ratio of disturbance sensitive to insensitive taxa.	No sign of alteration from undisturbed levels.	Slight alteration from type-specific levels.	Substantially lower than the type-specific level and significantly lower than for good status.
Level of diversity	No sign of alteration from undisturbed levels.	Slight signs of alteration from type-specific levels.	Substantially lower than the type-specific level and significantly lower than for good status.

1.2 Water Framework Directive and Acidification

Acidification is one of the key pressures covered by the WFD. Physico-chemical normative definitions (Table 1.2) are defined directly with regard to High status, but are otherwise dependent on the biological classification (Table 1.1).

Table 1.2 WFD Normative definitions for acidification-related physical-chemical quality elements relating to High Good and Moderate status.

Feature	High status	Good status	Moderate status
Level of pH, acid neutralising capacity etc.	Corresponds totally or nearly totally to undisturbed conditions. No sign of anthropogenic disturbance alteration from undisturbed levels.	Does not reach outside the range established so as to ensure the functioning of the ecosystem and the achievement of the values specified for biological quality elements.	Conditions consistent with the achievement of the values specified for biological quality elements.

2. ACIDIFICATION

2.1 Freshwater acidification in the UK

Acidification results from the atmospheric deposition of sulphurous and nitrogenous compounds, derived from industrial, vehicular and agricultural sources, and represents one of the most detrimental of anthropogenic impacts on upland freshwater ecosystems. Its effects are most pronounced in regions where geochemical weathering rates are relatively poor, including many upland areas in the west of Britain, southwest Northern Ireland and the Republic of Ireland where acidic peaty soils overlie poorly weatherable lithologies such as granites, sandstones and shales. Palaeoecological work has shown that many lakes (and by inference connecting streams) in these regions have acidified by up to 2 pH units since the onset of the industrial revolution as a direct result of acid deposition (Battarbee et al., 1990). Other effects on water chemistry include the mobilisation of biologically toxic labile inorganic aluminium (Al_{lab}), reduced availability of dissolved inorganic carbon (DIC) in the form of bicarbonate or dissolved carbon, the chronic depletion of concentration of base cations, such as calcium and magnesium and, possibly, the depleted availability of phosphorus. Recently it has also been shown that dissolved organic carbon (DOC) concentration is influenced by acid deposition (e.g. Evans et al., 2006) and it is likely that acidified lakes would often have exhibited substantially higher DOC levels prior to the onset of acidification. The UK Acid Waters Monitoring Network (AWMN) demonstrates that acidified waters have benefited from a substantial drop in the rate of sulphur deposition over the last two decades (Monteith and Evans 2005); severely acidified sites have shown reductions in Al_{lab}, while pH and alkalinity show increases in less acidic waters.

Acidification influences aquatic biota at all levels of the food chain, from primary producers, such as aquatic algae and macrophytes, to macroinvertebrates, fish and even water birds. Primary producers may be affected by the reduced availability of dissolved inorganic carbon (DIC – required for photosynthesis), macronutrients such as phosphorus, and changes in inter-specific competition. Aquatic animals are vulnerable to increased aluminium, hydrogen ion and heavy metal toxicity, and changes in food availability and quality. The AWMN has found evidence of recent changes in epilithic diatom, aquatic macrophyte, macroinvertebrate and salmonid populations which are indicative of improved chemical conditions (Monteith et al., 2005).

2.2 Acidification and macroinvertebrates

Macroinvertebrates are a particularly valuable biological group for bio-monitoring of aquatic systems due to their sensitivity to various physico-chemical stressors, ubiquity, local abundance, functional diversity and the relative ease with which species can be sampled and identified. Their use as indicators of water quality has been widely documented (see for example Rosenberg and Resh, 1993).

The relationship between macroinvertebrate community structure and the acidity of their aquatic habitat has been thoroughly investigated over the past three decades, although the predominant focus has been on running waters. Routine monitoring of macroinvertebrate communities in lakes has only recently become commonplace so it is rarely possible to demonstrate the nature of the biological response to acidification on a site specific basis. The primary source of information has come, therefore, from spatial studies of the relationship between species composition and acidity. On a broad acidity scale of, for example, pH 4.5 – 7.0, approximating to the full pH range over which sensitive sites in the UK may have acidified, relationships between macroinvertebrate community structure and acidity are very clear and may be summarised as follows:

a) Where species distributions are assessed with respect to a range of water quality parameters in acid sensitive systems, water pH (the inverse of the logarithm of hydrogen ion concentration (H^+)) is often identified as the chemical variable which explains the greatest amount of variance in the species data (Larsen et al., 1996; Davy-Bowker et al. 2003; Johnson et al., 2004).

b) Increased hydrogen ion concentration is thought to adversely affect osmoregulation in a number of macroinvertebrate species (Herrman et al., 1993). However, it is not clear to what extent the physiological effect of declining pH compares with co-varying factors including:
    - toxic effects of increasing aluminium solubility (which also interferes with osmoregulation);
    - the effect of iron and aluminium precipitates on feeding activity and oxygen uptake;
    - increasing toxic heavy metal solubility (e.g. cadmium, iron, lead and zinc);
    - indirect nutrient controls on food availability; etc.

    Indeed aluminium concentration, either represented by “total aluminium” or, more appropriately, Al_{lab} is also often found to be a strong predictor of the assemblage in spatial studies (e.g. Johnson et al., 2004) in addition to assessments of temporal variation in monitoring studies (e.g. Monteith et al., 2005).

c) Species show varying distributions across pH gradients, as illustrated, for example by Hämäläinen and Huttenen (1996) and Larsen et al., (1996). Certain species, including several molluscs, amphipods and mayflies, are confined to the less acidic end of the spectrum, whereas more tolerant species, including several stoneflies and chironomids are often present throughout much of the range. Few acid tolerant species are solely restricted to acidic sites.

d) Patterns are evident in ecological functioning across acidity gradients. Many acid tolerant species feed predominantly on detritus, although certain carnivorous taxa, such as water boatmen (Corixidae) and water beetles (e.g. Dytiscidae) may also thrive in the absence of acid sensitive higher predators such as salmonids. Specialised grazers feeding on attached algae (e.g. several mayflies) are often absent from more acidic sites. Some species previously assumed to be detritivorous may fill the niche of grazers in acidified systems and, therefore, may be considered more "generalist" in these circumstances (Ledger et al., 2005). While the relative balance of the assemblage may shift to one dominated by "collector-shredders" and predators with progressively more acid water, biological monitoring suggests that chemical recovery and consequent expansion of the aquatic food chain may encourage the recolonisation of other predators, such as dragonflies and caddisflies (Woodward and Hildrew, 2001; Monteith et al., 2005) and this could lead to an overall increase in predatory species in less acid environments.

e) The net effect of these relationships is a strong negative relationship between acidity and diversity parameters (see, for example, Petchey et al., 2004). There is evidence to suggest a logarithmic relationship between species richness (i.e. total number of species observed) and mean annual pH (up to a pH of circa 7.0), and this relationship does not appear to differ significantly between lake and stream systems (Woodward, pers. comm).

3. PHYSICO-CHEMICAL INDICATORS OF THE PRESSURE OF ACIDIFICATION

3.1 Distinguishing between the effects of Acidity and Acidification

If the relationship between the aquatic fauna and acidity is understood adequately it should be possible to predict the acidity of a site given the biological data. This forms the basis of the biological acidity-indicator systems discussed in further detail below. However, the remit of this project is to develop a tool with which to classify the biological impact of acidification, i.e. the extent to which the aquatic macroinvertebrate fauna has been affected by anthropogenically-driven change in acidity.

It is important, therefore to distinguish between the role of acidity in determining differences in biotic composition between sites, and the effects of the process of acidification on aquatic organisms and communities. pH *per se* is not an indicator of acidification; the pH of acid-sensitive lakes and streams in non-acidified regions (i.e. regions with low sulphur and nitrogen deposition) varies substantially depending on the amount of geological buffering and the contribution of organic acids from catchment soils.

In order for pH to be of value as a physico-chemical indicator under WFD60 it would first be necessary to estimate pH reference conditions for the sites in the study sites. This is feasible where palaeoecological information is available, since robust diatom-pH inference models are available with quantifiable and low levels of predictive error. However, there is currently an insufficient number of acid-sensitive lakes for which palaeoecological and macroinvertebrate data are available. At the same time, the ability of process-based physico-chemical models to predict pH is questionable as most do not account for possible links between changing acid deposition and organic acid solubility (see for example Battarbee et al., 2005). It is clear, therefore, that alternative physico-chemical metrics of acidification pressure are required for this project.

3.2 Acid Neutralising Capacity (ANC)

ANC is the preferred response variable in process-based acidification models concerned with Critical Loads. Representing the balance between base cations and strong acid anions, it is relatively easy to model (in comparison with pH) and has been shown to be a robust predictor of damage to salmonid populations (Lien et al., 1996). Relationships have also been shown for macroinvertebrates (Raddum and Fjellheim, 1984; Raddum and Skjelkvåle, 1995), and diatoms (e.g. Juggins et al., 1995). A negative ANC (i.e. a surfeit of acid anions over base cations) implies elevated concentrations of acid cations, i.e. hydrogen and aluminium ions, and hence acidic water. For water with a positive ANC, the surfeit of base cations may be accounted for by organic anions (i.e. DOC), bicarbonate and (at higher values) carbonate. In the field, ANC may fall during periods of high precipitation or snow melt as a result of dilution of high ANC groundwater or the delivery of mineral acids stored in the snowpack. ANC is also depleted during episodes of seasalt deposition, when marine cations temporarily displace hydrogen ions from soil exchange sites, particularly during winter storms.

ANC is normally determined as follows:

i.e. Ion balance ANC = $[Ca^{2+}] + [Mg^{2+}] + [Na^{+}] + [K^{+}]) - ([SO_4^{2-}] + [NO^{3-}] + [Cl^{-}]$

with all parameters expressed as equivalent concentrations.

However, Evans et al. (2001) suggested that this calculation was sensitive to the compound errors associated with the measurement of the 7 constituent ions, and that the resulting “noise” might hamper the detection of trends in ANC time series. An alternative expression of the same balance (Alkalinity-based, or Cantrell, ANC) is derived from Gran, or dual endpoint alkalinity, and organic acid (DOC) concentration. Here, an assumption is made regarding a standard charge per milligrame of DOC. In the UK the most commonly applied standard is 4.5 $\mu eq\ l^{-1}\ g^{-1}$ C for samples with a pH less than 5.5 or otherwise, 5.0 $\mu eq\ l^{-1}\ g^{-1}$ C. Recent AWMN data assessments include a further modification of the alkalinity-based expression to account for the influence of Al_{lab}. An assumption is made that all labile inorganic aluminium is trivalent and the conversion is 3 $\mu eq\ l^{-1}\ \mu g^{-1}$ Al_{lab}.

Theoretically these approaches should yield similar results although this is not always the case. Estimation of ANC from alkalinity, DOC and Al_{lab} is less sensitive to compound errors but carries uncertainty with regard to the amount of charge (protonation) attributed to DOC which is known to be pH dependent, and to a lesser extent Al_{lab}. Uncertainties associated with the ion balance method for estimating ANC should become less important where annual means are determined from a number of samples, as the effect of random errors should be dampened. Discrepancies between methods are currently under investigation by the AWMN.

Unlike pH, that has been proposed elsewhere as a possible physico-chemical standard for acidification pressure, ANC does convey information on the likelihood that a water body has been damaged by acidification. In upland acid-sensitive systems Cl^{-} is normally considered to be derived from charge-neutral sea salt while the other two strong acid anions SO_4^{2-} and NO_3^{-} largely reflect inputs from acid deposition. ANC thus reflects, predominantly, the capacity of base cation leaching to withstand the input of these latter two anions.

Natural waters almost invariably exhibit a surplus of base cations over strong acid anions, and waters with negative ANC are, therefore, very likely to have acidified. Low pH waters are common in unacidified regions where the influence of organic acids derived from organic soils is strong. However, ANC is normally positive in these systems, regardless of acid-sensitivity. Importantly for this project, Dangles et al. (2004) found diverse and functional macroinvertebrate communities in naturally acid (i.e. high DOC) waters but not in acidified waters of similar pH.

3.2.1 Estimating change in ANC using the Steady State Water Chemistry model

The steady state water chemistry (SSWC) model underpins UK critical load assessments and deposition scenario modelling. The Henriksen SSWC model formulation assumes that the greater the current base cation concentration:
i) the greater the likely source of bicarbonate weathering and ANC generation; and therefore,
ii) the lower the likelihood of acidification having taken place for a given deposition load.

SSWC uses measured SO_4^{2-} concentration as an index of deposition, on the assumption that this anion is mobile within catchments. The ratio of SO_4^{2-} to base cation concentration is used to determine what proportion of contemporary base cation concentration is due to acid deposition, and this is then used implicitly to back-calculate baseline ANC for an assumed pre-industrial sulphate concentration.

The key equation is:

$$[BC]_0^* = [BC]_t^* - F([NO_3^-] + [SO_4^{2-}]_t^* - [SO_4^{2-}]_0^*)$$

$[BC]_0^*$	pre-industrial non-marine base cation concentration
$[BC]_t^*$	current sum of non-marine base cations
F	correction factor: the "F-factor"
$[SO_4^{2-}]_t^*$	current non-marine sulphate
$[SO_4^{2-}]_0^*$	pre-acidification non-marine sulphate: "sulphate zero"

In the SSWC model the components of this equation are calculated as follows:

a) Current total non-marine base cations;

$$[BC]_t^* = [Ca^{2+}]^* + [Mg^{2+}]^* + [Na^+]^* + [K^+]^*$$

b) The F-factor;

$$F = \text{sine}\{\ 90[BC]_t^* / S\ \}$$

F is defined as the "change in base cation concentration per unit change in excess acid anions". It is a function of current base cation concentration and may vary for a given lake over a period of time. It is assumed that a high measured concentration of base cations in a lake indicates high "weatherability" of soils within the catchment and a large pool of exchangeable base cations in catchment soils.

S is an empirically derived value of $[BC]_t^*$ for which F=1; i.e. when current total non-marine base cations equal S, all acid deposition results in base cation leaching. Previous studies have found that S may vary from 200-400 μeql^{-1} from site to site, but that generally speaking, at S=400 $\mu eq\ l^{-1}$ the pH of the water will be in the range 6.5-7.0 and the leaching of base cations causes no change in pH. For critical loads work in the UK a value of S=400 μeql^{-1} is therefore used.

As a sine function the value of F can range from 0-1; in practice F ranges from near zero in dilute lakes to F=1 for lakes with high levels of base cations. Because F is a sine function there has to be a cutoff value of base cations above which F is taken to be 1; otherwise its value would start to decrease again at base cation levels greater than the constant S. In the SSWC model, F is therefore set to unity for any lake with $[BC]_t^* > 400\ \mu eql^{-1}$.

c) Sulphate zero;

The "background" concentration of SO_4^{2-} is taken as 15 µeq l^{-1}, the minimum figure observed in a study of near pristine Norwegian lakes (Braake, 1989), plus an extra component which is related to the "weatherability" of catchment soils and therefore proportional to [BC]t*. This figure has been derived empirically in other critical loads studies:

$$[SO_4^{2-}]_0^* = 15 + 0.16[BC]_t^*$$

When $[SO_4^{2-}]_t^* > 500$ µeql^{-1}, SO_4^{2-} is removed from the SSWC model calculation to avoid deriving improbably low critical load values for insensitive lowland sites. This means that base cation zero is taken to be the current total non-marine base cation value, i.e. $[BC]_0^* = [BC]_t^*$. This cutoff is intended to exclude catchments where SO_4^{2-} concentrations are too high to have been caused by atmospheric deposition alone.

It is assumed that pre-industrial nitrate concentration $[NO_3^-]$ in acid sensitive lakes was negligible, so that pre-industrial, baseline ANC can be calculated as:

$$ANC_0 = [BC]_0^* - [SO_4^{2-}]_0^*$$

The change in ANC according to the assumptions of the SSWC model can be calculated as the difference between current measured ANC and baseline ANC (ANC_0).

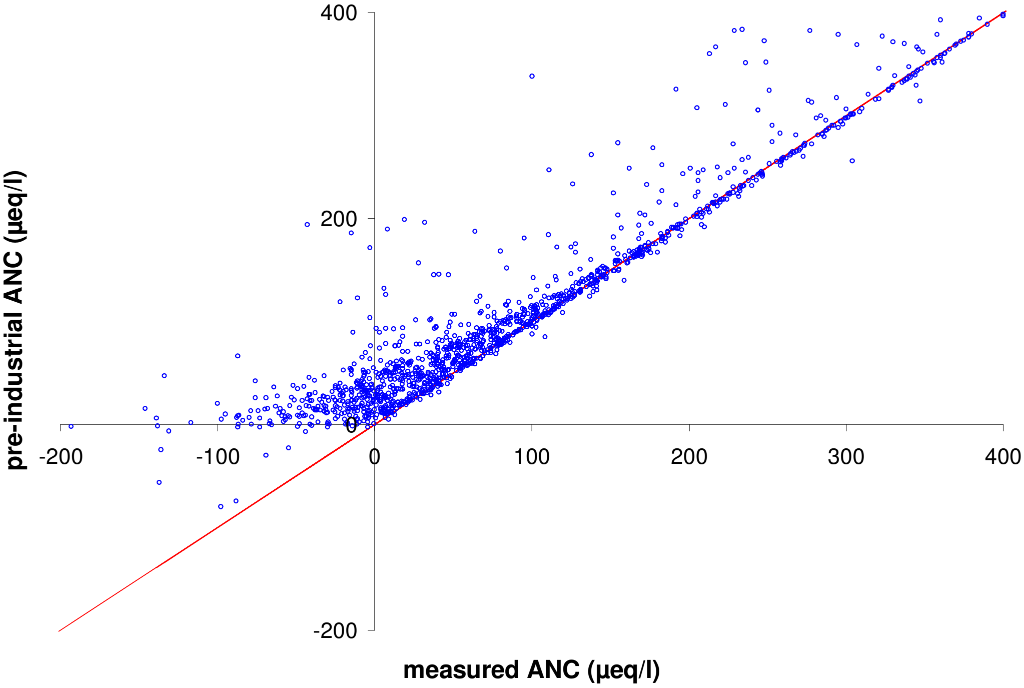
3.2.2 *ANC as a direct indicator of acidification pressure*

We applied the SSWC model to estimate ANC_0 for 830 UK lakes and streams with $pH < 7.0$ from the DEFRA Freshwater Umbrella database held at UCL. This demonstrated the relationship between contemporary chemistry and pre-industrial ANC, as determined by SSWC with its various assumptions, on a wide spatial basis.

Contemporary pH showed a relatively weak relationship with $ANC_{0.}$ Unsurprisingly however, given the model assumptions, the relationship between contemporary ANC and ANC_0 is much stronger (Figure 3.1). At the high ANC end of the plot the data largely fit the red 1:1 line, or show various degrees of positive deviation but no strong tendency for departure between current and pre-acidification ANC. With declining ANC the tendency for deviation from linearity increases. This plot demonstrates that, according to the SSWC model:

a) pre-industrial ANC would rarely have been negative, even for sites which are strongly negative ANC today;
b) there is little indication that sites with high ANC today (i.e. >100 µeq l^{-1}) would have had higher ANC in the past, i.e. these sites are unlikely to have acidified;
c) as ANC falls below 100 µeq l^{-1}, there is an increasing likelihood that a site would have had higher ANC in the past, i.e. that it will have acidified;
d) the likelihood of a site having experienced a large decline in ANC – e.g. 50 ueq/l – increases as contemporary ANC declines towards zero and beyond.

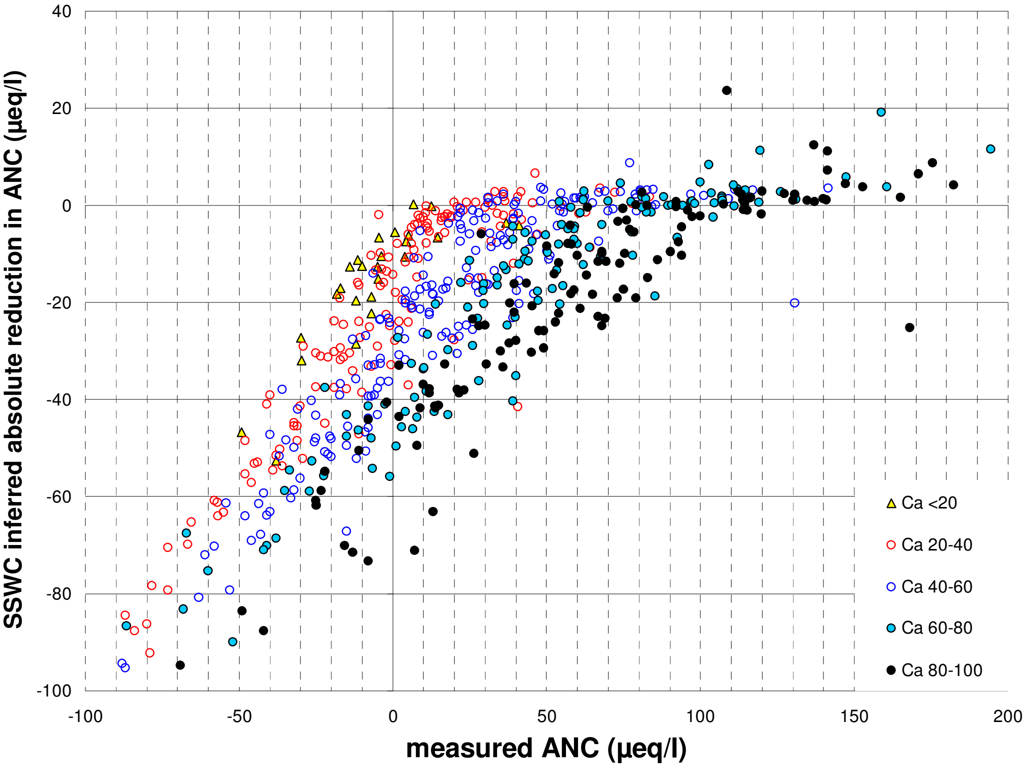
Figure 3.1 Relationship between contemporary ANC and SSWC inferred pre-industrial ANC based on 830 water samples from UK acid sensitive waters. Red line = 1:1.



Furthermore, the model implies that the amount of acidification predicted by the SSWC model for a given contemporary ANC is dependent on the contemporary base cation (e.g. Ca^{2+}) concentration. Figure 3.2 illustrates that, according to the SSWC model:

a) the extent to which ANC is predicted to have declined for a given contemporary ANC is positively related to contemporary Ca^{2+} concentration;
b) even at an ANC of 80-100 µeq l^{-1}, sites with a relatively high current Ca^{2+} i.e. (80-100 µeq l^{-1}) may have lost ANC - although this mostly equates to a less than 10% a reduction and is unlikely to be of great physico chemical or biological significance;
b) sites with a contemporary ANC as low as 10 µeq l^{-1} may not have acidified providing that the current Ca^{2+} concentration is very low (i.e. below 20 µeq l^{-1});
c) despite large variation in the acidification threshold between Ca^{2+} classes, all types of site with a current ANC of 0 µeq l^{-1} or less are modelled to have undergone a substantial reduction in ANC;
d) the discrepancy between Ca^{2+} classes in the amount of ANC change for a given current ANC is greatest at around 0 µeq l^{-1} and the discrepancy declines to negligible levels as current ANC approaches -100 µeq l^{-1}.

Figure 3.2 The amount of ANC reduction as a result of anthropogenic sulphur deposition (inferred by the SSWC model) related to current (measured) ANC. Data grouped into 5 calcium concentration classes (Ca^{2+} units µeq/l).



Thus, according to SSWC assumptions, the extent to which a site has acidified can be approximated from contemporary ANC in the context of the base cation concentration. Alternatively, the amount of deviation could be described in terms of acid anion concentration. Hence, sites with very low but positive ANC (i.e. approaching zero) and a low sum of concentrations of sulphate and nitrate may be unacidified, while sites with the same ANC but larger concentrations of sulphate and nitrate are more likely to have been impacted.

3.3 Labile inorganic aluminium

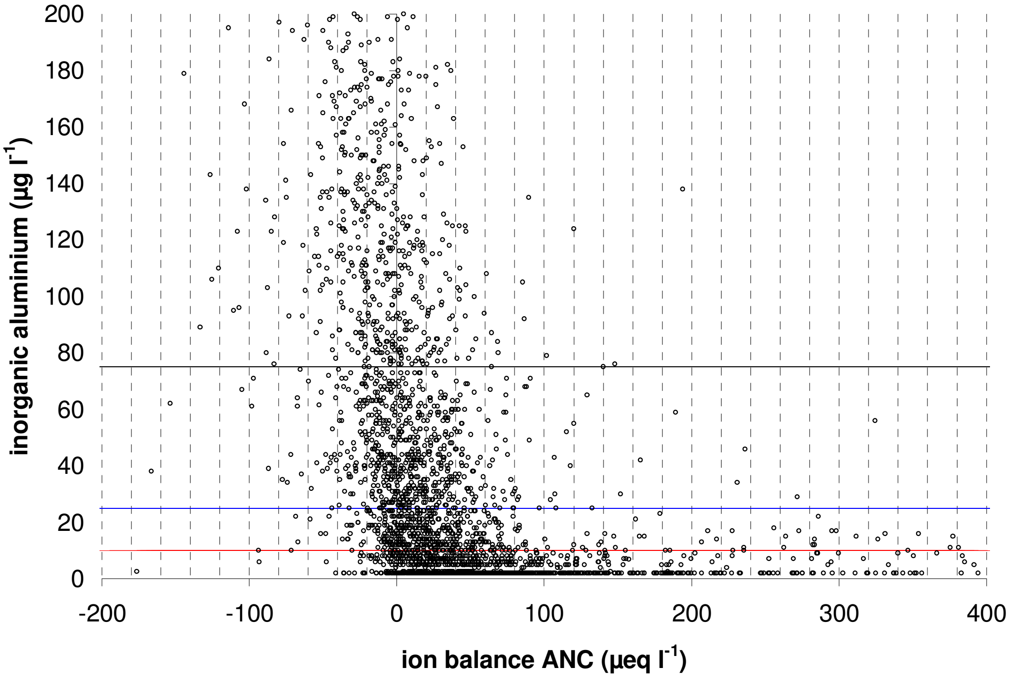
Labile inorganic aluminium is mobilised as soils acidify and is known to be highly toxic to many types of aquatic fauna. It has been shown to be the primary cause of salmonid death in Scandinavia (Rosseland et al., 1990) and is thought to exert a strong control on macroinvertebrate species composition. Rosseland et al. (1990) proposed that Al_{lab} became toxic to fish at concentrations between 25 and 75 µg l^{-1}. Recently, Al_{lab} was found to be the most important direct chemical predictor of change in AWMN macroinvertebrate communities (Monteith et al., 2005).

According to ECRC-ENSIS data holdings Al_{lab} concentrations rarely rise above 10 µg l^{-1} in UK waters which have not been acidified by acid deposition (rare exceptions include sites in catchments with unusual, sulphur-rich mineralogy, or exposed mine workings). In general terms, therefore, Al_{lab} concentrations of over 10 µeq l^{-1} in upland systems will normally be indicative of acidification and larger concentrations imply a greater likelihood of biological impacts.
Unfortunately, Al_{lab} is not routinely measured by the UK environment agencies and, beyond AWMN/ECRC-ENSIS data holdings (and additional data generated and held by the FRS

Laboratory, Pitlochry), we are not aware of other datasets (combined water chemistry and biology) that could be used for Al_{lab} calibration purposes in this project.

Broad classes of Al_{lab} concentration may be predicted on the basis of ANC. Figure 3.3 draws on all water chemistry samples collated for all AWMN sites. This shows that samples with an ANC above 40 µeq l^{-1} often have undetectably low Al_{lab} concentrations, and rarely exceed 10 µg l^{-1}. An ANC of circa 40 µeq l^{-1} might therefore be considered indicative of high status with regard to the physico-chemical normative definitions for the pressure of acidification through the mobilisation of aluminium. At the other extreme, water samples with an ANC below 0 µeq l^{-1} almost invariably have concentrations of Al_{lab} above the lower biological limit of 25 µg l^{-1} (Rosseland et al., 1990), and most samples with an ANC below -20 µeq l^{-1} have Al_{lab} concentrations above the higher limit of 75 µg l^{-1}. On physico-chemical evidence and published biological information, therefore, water with an ANC of less than 0 µeq l^{-1} might be considered to be in a condition ranging from “poor” to “bad”.

Figure 3.3 Relationship between labile inorganic aluminium concentration and ion balance ANC for all water samples in the UK Acid Waters Monitoring Network database (comprising 24 acid sensitive lakes and streams). Red line = 10 µg l^{-1} (theoretical maximum for unacidified UK waters); blue line and black line represent lower and upper limits for toxicological effects on fish (Rosseland et al., 1990).



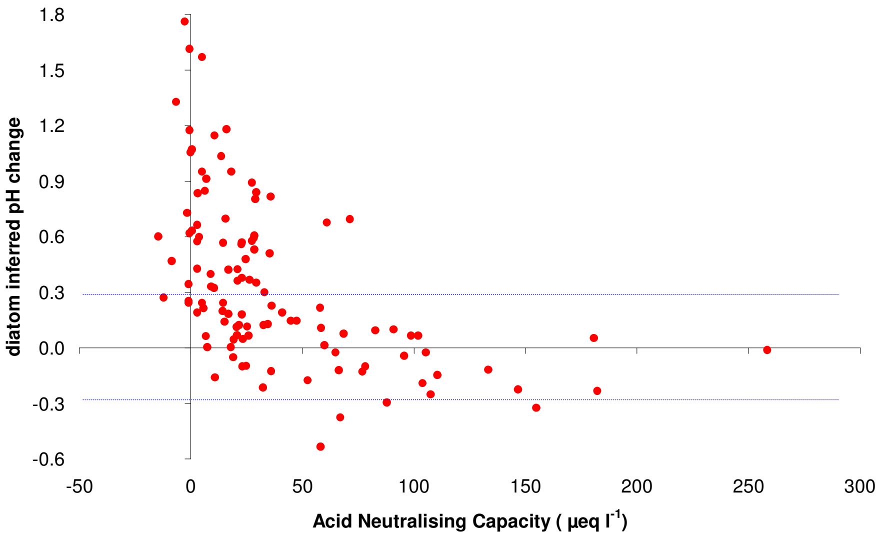
Recently, Lawrence et al. (2007) investigated the relationship between inorganic aluminium mobilisation and ion balance ANC with respect to DOC concentration for streams in the Adirondack Mountains, USA. They demonstrated that the point at which Al_{lab} concentrations become significant in an acidifying system equates to a “base cation surplus” (BCS) of 0 µeq l^{-1}, where BCS represents ion balance ANC minus the charge provided by DOC, using a single charge estimate of circa 6 µeq mg^{-1} C. In effect this implies that for waters with negligible DOC concentration (e.g. less than 1 mg l^{-1}) Al_{lab} will only become mobilised, and therefore of biological importance as ANC falls below 0 µeq l^{-1}. In waters with higher DOC, mobilisation will occur at higher ANC values. This observation should not be confused with the general observation that DOC offers some protection to aquatic biota at low pH. However, it again illustrates that ANC in isolation may not be sufficient to ascertain damage. For AWMN data a similar relationship can be demonstrated between Al_{lab} and ANC and Ca^{2+} concentration. In samples with Ca^{2+} concentrations below 20 µeq l^{-1}, Al_{lab} mobilisation is only apparent in waters at an ANC of around 0 µeq l^{-1} or below; whereas for the Ca^{2+} class of 60-80 µeq l^{-1}, mobilisation may occur at an ANC as high as circa 40 µeq l^{-1}.

3.4 Palaeoecological support for the use of ANC as an indicator of acidification

Some of the first work to reveal the geographical extent and timing of freshwater acidification in Scotland was based on the palaeoecological analysis of trends in diatom species composition in sediment cores (e.g. Flower and Battarbee, 1983). Freshwater diatoms are excellent indicators of lake acidity as most species occur only over very narrow pH ranges. The silicious outer-casing of diatoms preserves in lake sediments so it is possible to use changes in the relative abundance of species down a sediment core to infer how the pH of a lake has changed through time. Techniques for calibrating the diatom change to a pH change have developed from simple classification approaches, which grouped species into pH classes, to the more statistically rigorous weighted averaging or maximum likelihood methods (Birks et al., 1990). A crude but cost-efficient approach to estimate how much a lake has acidified is simply to determine the difference between the diatom inferred pH of the surface (i.e. modern) sample and a sample taken from circa 20 cm or more core depth, normally assumed (on the basis of sedimentation rates) to represent conditions prior to the onset of anthropogenic acidification in the mid 19th century.

Data provided by the DEFRA Freshwater Umbrella Programme demonstrate a striking relationship between diatom inferred pH change and current ANC (Figure 3.4). Given the model error of circa 0.3 pH units there is no obvious indication of acidification for sites with a current ANC of more than approximately 40 µeq l^{-1}. The relatively moderate inferred change in pH for some sites with negative ANC may reflect the increased role of aluminium as a buffer in these very acidic waters. These results are therefore fully consistent with physico-chemical observations discussed earlier with respect to the value of ANC as an indicator of acidification pressure.

Figure 3.4 Change in pH (as inferred from the difference in diatom inferred pH between samples from the top and bottom of sediment cores) in the context of contemporary ANC. Blue dotted lines indicate the Root Mean Square Error of Prediction for the weighted averaging based diatom pH transfer function.



3.5 Summary of physico-chemical indicators of acidification

In summary, both physico-chemical and palaeoecological models suggest that the relationship between ANC and Ca^{2+} may be used both to infer the likelihood and extent of lake acidification, and to predict the biologically crucial effect of elevated Al_{lab} concentration.

4. MACROINVERTEBRATES AS INDICATORS OF ACIDIFICATION

A principal challenge of this report is to relate WFD biological normative definitions for macroinvertebrates (Table 1.1) to physico-chemical indicators of acidification. A number of schemes and multivariate statistical techniques are currently available with which water acidity can be predicted on the basis of the macroinvertebrate community structure. These are summarised as follows.

4.1 Raddum Indices

The Raddum 1 Index (Raddum et al. 1988) is based on a simple classification system, whereby a range of taxa are ascribed to one of four classes (see Table 4.1) depending on their pH tolerance.

Table 4.1 Original classification framework defined by Raddum (1988)

Category	species tolerating pH	score
A	>5.5	1.00
B	>5.0	0.50
C	>4.7	0.25
D	<4.7	0.00

The community is then classified according to the highest scoring taxon present. Hence, the presence of one or more individual *Baetis* sp. (Score = 1) results in a classification of 1.0, i.e., the highest possible rating for the sample. If no species representing the first three classes is present the sample is rated as zero. The approach was developed by Fjellheim and Raddum (1990) to include more taxa. The Raddum I Index is routinely deployed in monitoring assessments such as the UNECE International Cooperative Programme on the Assessment of Acidification of River and Lakes Programme (e.g. Raddum, 1999). Application of the Index to the UK would require a revision of the taxon lists to allow incorporation of taxa not found in Scandinavia. While this system is logically based on tolerance limits and is simple to apply, it is likely to lack sensitivity, particularly in high DOC systems where reference condition communities might only be expected to classify between 0.25 - 0.50.

The Raddum II Index is normally only applied to river samples. This Index is based on the relationship between two groups of macroinvertebrates which show markedly different acidity distributions, i.e. the ratio of the total number of individuals of the mayfly genus *Baetis* sp. (a species of flowing waters) and the total number of individuals of acid tolerant stonefly species. A major limitation of both these schemes is that they are pH based and, for reasons outlined in Section 3.1, are not appropriate for the prediction of acidification pressure in landscapes where water chemistry is influenced strongly by organic acids

4.2 The Henriksson and Medin Index

This multi-metric approach is based on a large Swedish macroinvertebrate dataset from humic influenced rivers (Henriksson and Medin, 1986). The Henriksson and Medin Index integrates presence/absence of indicator taxa and ratios of sensitive to non-sensitive taxa and an estimate of species richness. The score for a sample is derived by calculating the sum of the score of 5 components representing: 1) the highest 0-3 Index score of a range of mayflies, stoneflies and caddisflies; 2) the presence/absence of amphipods (score 0 or 3); 3) the presence of sensitive groups (Hirudinea, Elmididae, Gastropoda and Bivalvia) (score 0-4); 4) the ratio of numbers of individuals of the Genus *Baetis* sp. to stoneflies (score 0-2); and, 5) the total number of species in a comprehensive list of 517 aquatic macroinvertebrate species (score 0-2). The criteria above are highly compatible with the major requirements of WFD Normative definitions, i.e. with regard to the need to use information on taxonomic composition and abundance (1 & 2), ratios of disturbance sensitive to insensitive taxa (4), presence/absence of major taxonomic groups (1,3 & 5) and estimates of levels of diversity (5). It has been proposed that an Index of 6.0 represents a threshold below which the probability of effects of acidification are "likely".

The Henriksson-Medin Index has been demonstrated by Johnson *et al.* (2004) to correlate with the pH and ANC of 48 lakes in a mixed forest ecoregion of Sweden. They observed that the Index showed a "funnel-shaped" relationship with these acidity variables and proposed that the reduction in variance about the regression lines with increasing acidity was indicative of increased acid stress. Classification of class boundaries was based on the extent of this variance and resulted in five classes for pH (<5.0, 5.0-5.6, 5.6-6.2, 6.2-6.8 and >6.8) representing extremely acid, very acid, acid, weakly acid and neutral-alkaline lakes respectively. The "acid" class was defined by the first obvious increase in residual variance and its upper boundary was selected to intersect with an Index of 6.0.

Four classes were defined for the relationship with ANC. All sites with an ANC < 20 µeq l^{-1} showed little residual variance, had Index scores below 3.0 and were deemed to be in the most acid class. This, they argued, was consistent with the findings of Lien et al. (1996), which suggested that an ANC 20 µeq l^{-1} represents a significant tolerance level for fish. Once again an Index of 6.0 was used to define the lower boundary for the non-acid class, corresponding approximately to an ANC of 150 µeq l^{-1}.

Johnson et al. (2004) found that, in contrast with pH and ANC, relationships between the Index and Al_{lab} concentrations were non-linear. However there were clear patterns in the data. Almost all sites where Al_{lab} concentration was below 20 µg l-1 had relatively high Henriksson - Medin scores (i.e. >5.0). This was taken as the threshold below which aluminium effects would be low and is consistent with observations of Rosseland et al. (1990) that concentrations of less than 25 µg l^{-1} have negligible effects on aquatic biota. All sites but one, which lay above the upper threshold of 75 µg l-1 (Rosseland et al., 1990), had scores of around 5.0 or less. These findings are particularly interesting, given the comments in Section 3.3, and suggest that this Index may have value as an indicator not only of acidity but also acidification status. On the basis of the observations for aluminium concentration, however, a Henriksson - Medin score of 5.0 might be a more appropriate threshold for good status than the more conservative value of 6.0.

4.3 AWIC – Acid Water Indicator Community

The AWIC, or Acid Water Indicator Community, classifications were developed by staff at the Centre for Ecology and Hydrology, Dorset, primarily to assist the UK Environment Agency in their assessment of the extent of ecological damage caused by the acidification of running waters. Two classifications, for family level and species level data, were based on an extensive biological and chemical database (487 samples, 410 sites) drawn from several regions of England and Wales (Davy-Bowker et al., 2003; Davy-Bowker et al., 2005).

Both classifications are based on partial Canonical Correspondence Analysis (pCCA) in which biological data are constrained by mean pH (based on a minimum of 5 samples taken over three years), with significant physical factors such as altitude and slope included as covariables.

The first axis scores for each taxonomic group are then allocated to one of six “bins” depending on their relative position on this axis. The sample score is determined according to an average score per taxon method (ASPT), termed AWIC (fam)–ASPT or AWIC (sp)–ASPT, for the family and species classifications respectively. Initial testing of the AWIC (fam)–ASPT approach (Davy-Bowker et al., 2003; Ormerod et al., 2006), using a “partially independent” dataset demonstrated that this Index is strongly correlated with pH. However, the relationship is heavily influenced by sites with a mean pH greater than 7.0 (which form the vast majority of the dataset). For sites with pH <7.0 the relationship shows considerable scatter. While the approach, at least at the species level, has potential for inferring the mean pH of a system, it does not allow for the differentiation of acidified and naturally acidic waters which is central to the WFD60 project.

4.4 Weighted Averaging based approaches

Being based on CCA, the methods applied in the AWIC classification are related to Weighted Averaging (WA) regression, a commonly used environmental diagnostic procedure which has been found to perform particularly well in diatom-pH calibration exercises (used to infer pH from fossil remains in sediment cores). In essence, weighted averaging is used to determine the optimal value of an environmental variable for individual species, and then the abundance weighted average of the optima of all species present in a sample is used to infer that environmental variable for a given site. The predictive error of the method may be assessed using “bootstrapping” procedures, in which individual samples are taken from the dataset to test the predictive power of models based on the remaining data.

Weighted averaging was used to investigate the relationship between macroinvertebrate assemblages and stream minimum pH by Hämäläinen and Huttenen (1990). They compared it with a “Tolerance limit” approach (TL), the tolerance limit of individual species being defined by the lowest pH of water in which each occur. Hämäläinen and Huttenen (1996) found that WA performed better than TL, resulting in lower root mean squared error of prediction. Larsen et al., (1996) also demonstrated the power of the WA approach for Norwegian streams, and showed that macroinvertebrates are as good predictors of pH (Root Mean Standard Error of Prediction for WA = 0.309 pH units) as more conventionally used diatoms. In their assessment of species distributions across the pH gradient they found a variety of patterns (i.e. unimodal, sigmoidal or indicative of either high or low pH) suggesting that a combination of Gaussian regression and direct gradient analysis might be necessary to provide a complete overview of indicator taxa.

4.5 Diversity based indices

It is widely recognised that environmental stress influences species diversity, a term often taken to encapsulate information both on the total number of species (species richness) and their relative distribution (or evenness). Species richness *per se* has been shown to increase across a gradient of pH in acid sensitive systems for a range of trophic levels (Petchey et al., 2004). However, estimation of the true number of species in a population of interest is subject to problems of rarefaction, i.e. the number of species in a sample will be dependent on the size of the sample taken. Possibly one of the most robust and widely used diversity indices is Shannon’s Index, defined as the sum of the product of the proportional abundance of each species and its natural logarithm, converted to a positive number by a prefixed negative sign. This can be translated into more meaningful values by determining its exponent (Hill’s N1; Hill, 1973). Hill’s N1 represents the “effective number” of abundant species in a sample and is thus more readily interpretable than the original index.

An alternative and commonly used diversity index, which does not include information on species proportions, is Margalef’s Index, defined as the number of species divided by the natural logarithm of the total number of individuals. This makes the assumption that species proportions will become less even as the total abundance of individuals increases. Resh and Jackson (1993) found that this was the only community-based index which showed a significant response in macroinvertebrates to an acid impact.

4.6 The need for a new macroinvertebrate-based acidification tool under WFD60

While most classification schemes reviewed above illustrate the potential for macroinvertebrate communities to predict the acidity of a freshwater system, few meet the fundamental WFD classification requirement to infer the pressure of acidification. The Henriksson Medin Index is the only system which shows real potential in this respect, since relationships with ANC and Al_{lab} have been demonstrated explicitly. However, the information necessary for us to assess underlying model assumptions and the relative importance of each component are not available, and we are unclear about the necessity of the current complexity of the model. Ideally the WFD60 tool should be based on as simple a model as possible, to minimise potential complications resulting from variation in sampling effort and taxonomic skill. Furthermore the Henriksson Medin Index is specifically calibrated for the Swedish boreal eco-region. There is a clear need, therefore, for the development of a new UK-based classification procedure under WFD60, and this requires a new physico-chemical/biological database to cover acid-sensitive UK lakes.

5. PRELIMINARY DATA ASSESSMENT

A central part of the WFD60 project was the collation of a database to contain the biological and physico-chemical information necessary to build and test the tool. At the outset it was envisaged that a substantial database, comprising several hundred macroinvertebrate and water samples, would result.

5.1 Data sources

Five main sources of data have been used within the WFD60 project

1) A lake macroinvertebrate – water chemistry dataset, derived from data from projects funded by DEFRA, including the AWMN, and currently held in databases at ECRC-ENSIS;
2) Macroinvertebrate data generated in variations to the WFD60 contract and based on samples collected by SEPA between 2003-2006
3) Water chemistry data for lochs in Galloway provided by the FRS Freshwater Laboratory, Pitlochry.
4) Water chemistry data from the SEPA lake water chemistry database to accompany biological data collated under point 2.
5) Macroinvertebrate and water chemistry data for lakes from the Environment Agency of England and Wales.

Only macroinvertebrate and water chemistry data that could be applied to specific components of the calibration exercise were included in the final WFD60 database. A substantial amount of SEPA macroinvertebrate data could not be used in this project since the bulk of this was collected in Autumn, whereas the dependency of this project on establishing links between water chemistry and ECRC-ENSIS data holdings has meant that the focus must be on Spring samples.

5.2 Quality and screening of the macroinvertebrate – water chemistry dataset

Protocols for macroinvertebrate sample collection and the water chemistry sampling and analysis differ inevitably between the projects and programmes outlined in Section 5.1. We have settled for “lowest common denominator” criteria as follows:

- Macroinvertebrate data must be derived from kick samples from lake stony littoral habitats using a standard long-handled pond net. Each kick sample must be of at least of one minute duration. Ideally more than one sample, from separate locations, should be taken to represent a lake on any sampling visit;

- Macroinvertebrate data must represent a full count, or at least an estimate, of all individual animals in the sample identified to Mixed Taxon Level;

- Owing to the relative paucity of macroinvertebrate data collected in Autumn that could be related to the full required suite of water chemistry data, WFD60 focuses on Spring sampled macroinvertebrates only. The Spring season was deemed to extend into June for sites in northern Scotland. Samples were thus to be collected between February and the first 10 days of June.

- Owing to considerable problems with the quality of water chemistry data provided by the environment agencies, the range of essential chemical determinands (in the final classification exercise) was restricted to the following:

  - pH
  - dissolved organic carbon (DOC)
  - calcium (Ca^{2+})
  - conductivity
  - ANC (determined either by ion balance or the Cantrell method depending on the availability of constituent data)

  (Cantrell ANC (μeq l^{-1}) = Gran Alkalinity (μeq l^{-1}) + f*DOC (mg l^{-1}) (where f = 4.5 for samples with pH < 5.5, and 5.0 when pH > 5.5)

- Sufficient water samples were required to allow the estimate of annual mean chemistry over any one year period, within one year (prior to or after) the collection of the macroinvertebrate sample. A series of water samples taken prior to the collection of the macroinvertebrate sample was preferred. Again, due to the paucity of data of acceptable quality, as few as three samples were accepted for the estimation of an annual mean. Where five or more samples were available these had to be distributed approximately evenly within the course of one year.

Despite relatively modest compliance requirements our final dataset, comprising Spring sampled macroinvertebrate data and matching mean annual water chemistry, consisted of only 107 sites. Due to concerns that this rather small number of samples might restrict model development we compiled a second dataset based on Spring sampled water chemistry only. In this second dataset we included sites represented by one Spring water chemistry sample only although if more data were available within this season then a mean value was determined. This resulted in a small increase in the number of sites to 120. However, preliminary data analysis suggested relatively poor relationships between the macroinvertebrate assemblages and water chemistry, possibly due to problems presented by short-term variability in water chemistry. Consequently we were unable to develop this further.

5.3 The WFD60 database

The data used in this project are stored in a Microsoft Access relational database housed at ECRC-ENSIS. Due to several concerns with water chemistry data quality from different sources, the database is built around the available macroinvertebrate samples. Chemistry data (for the determinands listed in Section 5.2) are only included in the chemistry data tables provided there are sufficient measurements to meet the annual mean estimate requirements for specific macroinvertebrate samples in the database. All macroinvertebrate data generated through WFD60 contract variations are included whether or not there is sufficient supporting water chemistry. Macroinvertebrate samples which do not have sufficient supporting chemistry are used at the end of the project to test the WFD60 tool (with respect to geographic distribution of lake classes). The database also includes tables providing information on macroinvertebrate and water chemistry samples (e.g. provenance, sample date, etc.), a table detailing geographic information on sites, a “species dictionary” which relates species names to macroinvertebrate Furse codes, and a series of Access queries enabling the determination of annual average water chemistry, selection of appropriate macroinvertebrate sample data and the generation of biological summary statistics.

5.4 The interim dataset

At the onset of the WFD60 project we explored the relationship between macroinvertebrate community structure for a wider range of physico-chemical variables than were available for later stages of the project. These data were drawn from ECRC-ENSIS data holdings and comprised 38 sites (described from now as the Interim dataset). The macroinvertebrate samples for these sites were all taken during Spring. While these data are of high quality, the relatively low number of samples restricts the potential power of the resulting analyses. Details of the samples included in the Interim dataset are provided in Appendix 1.

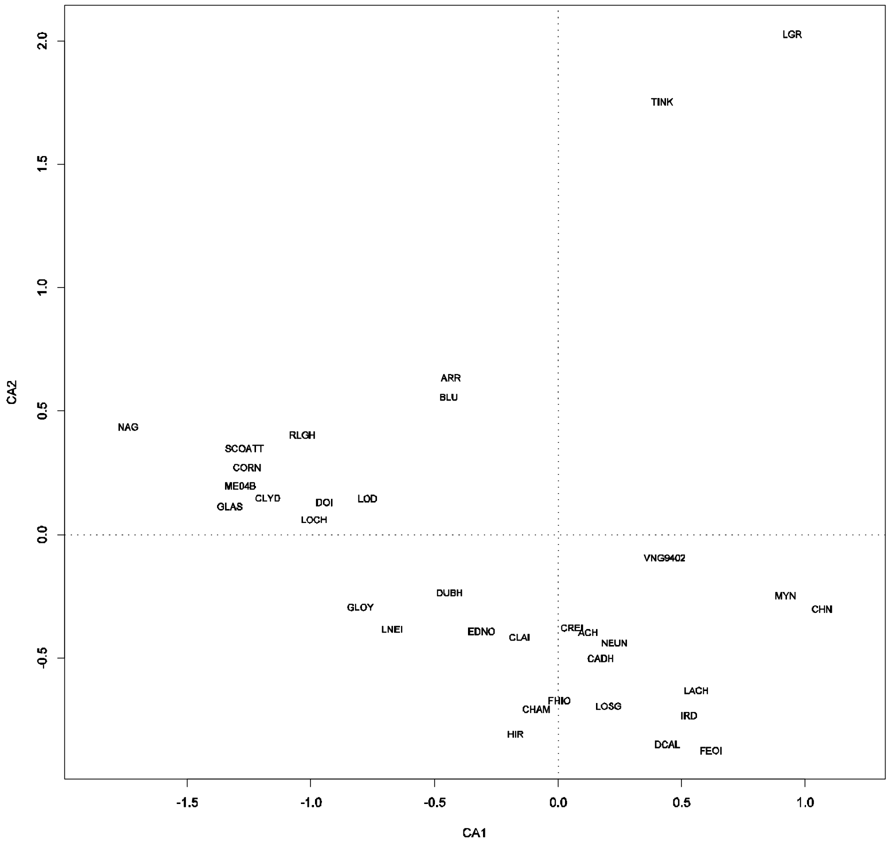
5.4.1 Indirect ordination

All multivariate analyses were conducted using the vegan ecological statistics package (Oksanen et al., 2007) in the R statistical software package (R Core Development Team, 2007).

Macroinvertebrate data, in the form of raw counts, were first ordinated by detrended correspondence analysis (DCA). The gradient length was approximately 3.0 indicating that unimodal rather than linear techniques were most appropriate for subsequent data analysis.

Correspondence Analysis (CA) revealed three major outliers, Burnmoor Tarn, Llyn Llagi and Llyn Cwellyn, resulting from the occurrence of small numbers of individuals of a limited number of taxa which were found at no other sites. Since these had a disproportionate influence on the ordination the sites were removed from this analysis. A CA ordination plot of site scores for the modified dataset is presented in Figure 5.1. This shows a satisfactory distribution of sites across the first two CA Axes. High elevation sites, such as Lochnagar (NAG), Scoat Tarn (SCOATT) and Llyn Glas (GLAS) cluster in the upper left of the plot but otherwise there is no broader indication of an influence of altitude on the ordination of sites on these axes.

Figure 5.1 Correspondence Analysis (CA) of macroinvertebrate assemblages for 35 acid-sensitive UK lakes



5.4.2 Direct ordination with chemical variables

The macroinvertebrate data for the 35 remaining lakes were then subjected to Canonical Correspondence Analysis (CCA) with the chemical parameters listed in Table 5.1 available as explanatory variables. CCA derives a set of ordination axis scores for species and samples. For the first axis, species scores and sample scores are chosen to maximise the correlation between them. Scores on subsequent axes are also maximally correlated, but uncorrelated with species and sample scores of the previously derived axis. In the following analyses all chemical data were standardised.

First, CCA was performed for each chemical variable individually, to determine the maximum amount of variance each could explain, regardless of potential covariant effects.

Table 5.1. Variance of the 35 lake macroinvertebrate dataset explained by chemical variables applied individually in Canonical Correspondence Analysis (CCA). P-value determined by Monte-Carlo permutation test.

Variable	**% total variance explained**	**p-value (1000 permutations)**
H^+ (hplus)	7.91	0.017
Alkalinity (alk)	5.73	0.019
Conductivity (cond)	3.66	0.284
calcium (Ca)	6.66	0.001
magnesium (Mg)	4.08	0.210
potassium (K)	3.80	0.235
nitrate (NO3)	6.83	<0.001
sulphate (SO4)	3.29	0.337
labile inorganic aluminium (labileAl)	9.01	0.006
dissolved organic carbon (DOC)	8.48	<0.001
ion-balance ANC (ionANC)	7.82	<0.001

Hydrogen ion, alkalinity, calcium, nitrate, Al_{lab}, DOC and ion-balance ANC all showed significant ($p<0.05$) relationships with the species data. DOC, ion-balance ANC and nitrate concentration were most highly significant, while Al_{lab}, followed by DOC, explained the largest amount of the variance. Ion-balance ANC and hydrogen ion concentration explained very similar amounts of variance (approximately 8%).

CCA was then repeated to include all the above as explanatory variables. In this analysis the variables explained 47 % of the total variance in the species data. 22.4 % of the total variance was accounted for in the first two axes of the ordination (see Table 5.2).

Table 5.2 Summary statistics for CCA of macroinvertebrate assemblages for 35 acid-sensitive UK lakes. Mean squared contingency coefficient = 3.528.

CCA Axes	**1**	**2**	**3**	**4**
Eigen values	0.4543	0.3372	0.2139	0.1809
Cumulative variance of species data	0.1288	0.2244	0.2850	0.3363

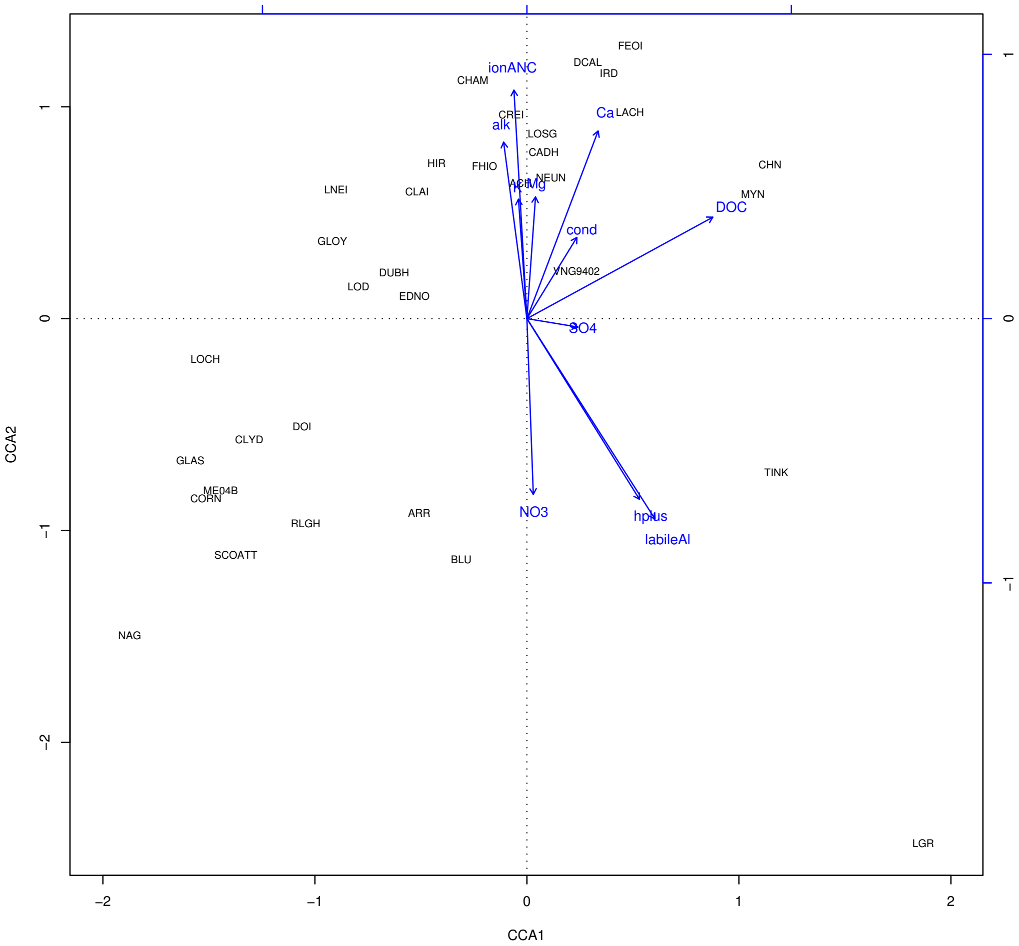
Biplot scores (correlations between ordination axes and environmental variables) are provided in Table 5.3. The first axis was related predominantly to DOC, while there was also some interaction with hydrogen ion and Al_{lab}. The second axis appeared to represent the main acidity axis with a particularly strong correlation with ion-balance ANC in addition to hydrogen ion and

Al_{lab}. Nitrate was also strongly correlated with this axis, suggesting that this anion, rather than sulphate, may exert the more important anthropogenic acidity effect on this small dataset.

Table 5.3 Biplot scores for chemical variables and ordination axes for the CCA of macroinvertebrate assemblages for 35 acid-sensitive UK lakes

	CCA1	CCA2	CCA3	CCA4
H^+	0.43	-0.68	-0.05	-0.37
alkalinity	-0.09	0.67	0.17	0.25
conductivity	0.19	0.31	0.05	-0.19
Calcium	0.27	0.71	-0.05	0.01
magnesium	0.03	0.46	0.07	-0.22
potassium	-0.03	0.45	0.14	-0.19
Nitrate	0.02	-0.66	-0.46	0.36
sulphate	0.19	-0.03	-0.33	-0.13
labile inorganic aluminium	0.48	-0.76	0.00	-0.18
dissolved organic carbon	0.70	0.38	-0.02	-0.23
ion balance ANC	-0.05	0.86	0.13	-0.19

Figure 5.2 CCA Ordination plot for Axes 1 and 2 of the macroinvertebrate – water chemistry dataset for 35 acid-sensitive UK lakes



Species scores, which describe the extent of the correlation of individual species with the ordination axes, are given in Table 5.4. Several species showed relatively extreme scores on both axes 1 and 2, possibly representing influences of organic and mineral acidity respectively. The stoneflies *Leuctra inermis* and *Nemoura* sp., (known for their acid tolerance) showed strong positive scores on Axis 1 (associated with high DOC) and strong negative scores on axis 2 (associated with low ANC). The same relationships were observed for the caddisflies *Oecetis ochracea* and *Anabolia nervosa* and the beetle *Oulimnius tuberculatus*. The corixids *Sigara dorsalis* and *Callicorixa wollastoni*, on the other hand, only showed strong (negative) scores with Axis 2 indicating these species are most abundant in relatively clear acidified systems. The abundance of these predators may be indicative of the absence of fish (perhaps lost through acidification) in these lakes

In contrast to the observations above, few species showed both strong negative Axis 1 scores and strong positive Axis 2 scores. Species with associations with low DOC sites included several which are commonly associated with “non-acidified” systems, including the mayflies, *Ecdyonurus* sp. and Siphlonuridae, and the limpet *Ancyclus* sp.. Species associated with high ANC lakes included the acid sensitive stonefly *Isoperla grammatica* and the mayfly family Baetidae.

This analysis on a small but high quality interim dataset therefore provided evidence for:

i) strong acidity controls on the macroinvertebrate dataset;
ii) differentiation between species indicative of acidified rather than naturally acid lakes;
iii) Al_{lab} as a potentially important indicator of species composition;
iv) ANC to be at least as powerful a predictor of macroinvertebrate assemblage structure as hydrogen ion concentration (or pH).

Table 5.4 Macroinvertebrate species scores for the CCA of macroinvertebrate assemblages for 35 acid-sensitive UK lakes. Data are sorted by the score on the second CCA axis, deemed to be the dominant acidity axis and particularly strongly correlated with ANC.

Taxon	Axis 1	Axis 2	Axis 3	Axis 4
Oecetis ochracea	1.77	-2.30	-0.24	-0.95
Oulimnius tuberculatus	1.70	-2.14	-0.17	-0.87
Agrypnia obsoleta	1.40	-1.93	-0.09	-0.64
Callicorixa wollastoni	0.53	-1.92	0.41	-0.16
Anabolia nervosa	1.53	-1.78	-0.18	-0.61
Deronectes griseostriatus	0.80	-1.77	0.40	-0.42
Leuctra inermis	1.62	-1.76	-0.21	-0.54
Agabus chalconotus	-0.03	-1.71	1.21	0.25
Sigara dorsalis	-0.03	-1.71	1.21	0.25
Nemoura sp.	1.31	-1.60	-0.12	-0.35
Agabus bipustulatus	-0.68	-1.59	0.46	0.55
Capnia	-1.57	-1.08	-2.48	1.15
Oreodytes davisii	-1.57	-1.08	-2.48	1.15
Baetis sp.	-0.32	-0.98	2.92	2.03
Centroptilum pennulatum	-0.32	-0.98	2.92	2.03
Rhithrogena semicolorata	-0.32	-0.98	2.92	2.03
Hydroporus palustris	-0.67	-0.87	0.27	0.26
Diura bicaudata	-1.46	-0.80	-1.24	0.84
Halesus	-0.24	-0.76	0.19	0.32
Leuctra hippopus	-0.58	-0.74	-0.05	0.55
Glaenocorisa propinqua	0.67	-0.66	0.17	0.73
Agrypnia varia	0.67	-0.62	0.11	-0.05
Annelida (Oligochaeta)	1.04	-0.58	0.61	0.09
Chaetopteryx villosa	-0.58	-0.57	0.94	-0.38
Cordulegaster boltonii	-0.86	-0.57	0.26	-1.17
Chaoboridae	-0.34	-0.57	0.54	-0.03
Polycentropus sp.	0.12	-0.55	0.94	0.05
Limnephilus sp.	0.90	-0.54	0.28	0.54
Oreodytes sanmarkii	-1.56	-0.54	1.21	-0.63
Chloroperla	-1.14	-0.48	0.17	0.73
Hesperocorixa castanea	-0.20	-0.47	-0.39	1.18

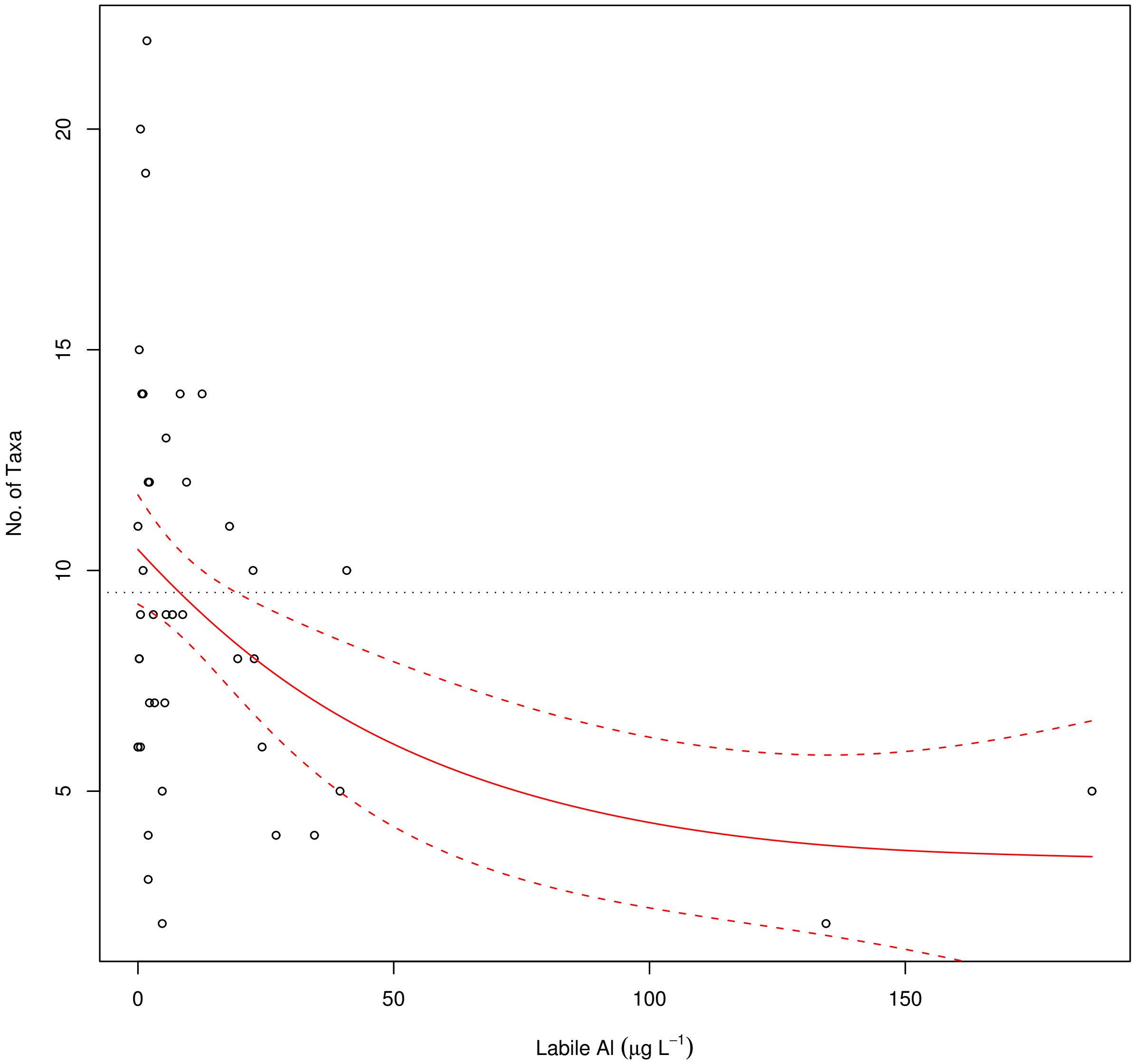
Ancylus sp.	-1.54	-0.46	-0.43	0.64
Asellus aquaticus	-1.54	-0.46	-0.43	0.64
Ecdyonurus sp.	-1.54	-0.46	-0.43	0.64
Potamophylax latipennis	-1.54	-0.46	-0.43	0.64
Plectrocnemia sp.	0.25	-0.46	0.22	0.65
Empididae	-0.88	-0.43	1.19	0.29
Chironomidae	-0.68	-0.36	-0.07	0.03
Tipulidae	-0.53	-0.36	0.19	0.45
Cyrnus	-0.24	-0.29	0.78	0.13
Siphlonuridae	-1.41	-0.29	1.64	0.17
Halesus radiatus	-1.17	-0.28	0.67	-0.42
Cordulia	1.35	-0.22	0.32	1.45
Erpobdella octoculata	1.35	-0.22	0.32	1.45
Mesophylax impunctatus	1.35	-0.22	0.32	1.45
Aeshna	-0.43	-0.18	0.17	-0.20
Nemurella picteti	-0.65	-0.17	-0.41	0.21
Agabus arcticus	-0.60	-0.15	0.21	-0.21
Hygrotus novemlineatis	0.17	-0.14	-0.43	1.29
Arctocorisa germari	0.96	-0.12	-0.30	1.02
Polycentropodidae	-1.06	-0.11	-0.13	0.24
Amphinemura sulcicollis	-0.15	-0.11	1.52	-0.13
Limnephilidae	-0.69	-0.11	-0.05	0.06
Simuliidae	-0.31	-0.11	-0.73	0.11
Holocentropus sp.	1.28	-0.10	0.19	1.46
Plectrocnemia conspersa	-0.94	-0.04	-0.05	-0.05
Haliplidae	-1.01	-0.04	0.72	0.32
Tubificidae	-0.87	-0.03	1.62	0.00
Centroptilum luteolum	-0.37	-0.01	1.38	0.62
Enchytraeidae	-0.98	-0.01	1.44	0.27
Deronectes depressus	0.84	0.00	-0.03	1.15
Dicranota sp.	-1.02	0.02	0.18	-0.17
Capnia bifrons	-0.68	0.02	0.60	0.53
Sigara scotti	1.21	0.03	0.05	1.47
Brachycentrus subnubilus	1.11	0.03	0.89	0.49
Lymnaea peregra	-0.29	0.04	0.63	0.08
Pisidium sp.	0.20	0.05	0.41	-0.03
Chloroperla torrentium	-0.76	0.05	0.01	0.17
Psychomyiidae	-0.95	0.05	-0.61	-0.19
Siphlonurus lacustris	-0.20	0.07	1.28	0.40
Collembola	-0.86	0.08	-0.45	-1.41
Cordulegasteridae	-0.86	0.08	-0.45	-1.41
Triturus sp.	-0.86	0.08	-0.45	-1.41
Ceratopogonidae	-0.07	0.09	0.69	0.28
Nemoura cambrica	-0.37	0.13	0.32	-0.60
Deronectes	-0.98	0.13	0.55	-0.71
Caenis moesta	-0.04	0.14	-0.47	1.18
Leuctra nigra	0.53	0.16	0.03	0.96
Plecoptera	-0.05	0.19	0.17	0.22
Agrypnia picta	-0.56	0.26	-0.20	-1.02
Pyrrhosoma nymphula	1.06	0.28	-0.23	1.49
Hydracarina	-0.65	0.28	0.36	-0.25
Anisoptera sp.	-0.21	0.29	0.27	-0.54
Diptera	-0.46	0.30	0.33	-0.35
Cymatatia bonsdorffi	1.04	0.32	-0.26	1.50
Elmis aenea	-0.53	0.33	1.88	0.03
Erythromma	-0.53	0.33	1.88	0.03
Rhabdiopteryx acuminata	-0.53	0.33	1.88	0.03
Siphlonurus armatus	-0.53	0.33	1.88	0.03
Trichoptera	-0.23	0.33	0.22	0.09
Glossiphoniidae	-0.11	0.34	0.43	0.31
Siphlonurus sp.	-0.52	0.34	1.83	0.01
Mystacides sp.	0.85	0.34	0.84	0.25
Dytiscidae	-0.30	0.35	0.53	-0.31
Agrypnia sp.	-0.23	0.37	0.13	-0.60
Leptophlebiidae	0.52	0.37	-0.16	0.22
Polycentropus flavomaculatus	-0.47	0.38	-0.08	-0.23
Limnephilus lanatus	-0.53	0.41	1.53	-0.18
Lepidostoma hirtum	-0.48	0.42	1.54	-0.32
Coleoptera	-0.32	0.43	0.42	-0.60
Naididae	-0.10	0.45	0.21	-0.19
Oulimnius sp.	-0.16	0.46	0.64	-0.33
Athripsodes sp.	0.69	0.49	-0.37	0.97
Leptophlebia marginata	-0.42	0.49	-0.34	-1.22
Zygoptera	-0.37	0.50	-0.48	-1.43

Sialis lutaria	0.43	0.50	-0.21	0.19
Heptageniidae	-0.14	0.51	1.11	-0.11
Lumbriculidae	-0.04	0.53	0.46	-0.20
Callicorixa praeusta	0.92	0.53	-0.50	1.52
Deronectes assimilis	0.92	0.53	-0.50	1.52
Gyrinus aeratus	0.92	0.53	-0.50	1.52
Hesperocorixa sahlbergi	0.92	0.53	-0.50	1.52
Ischnura elegans	0.92	0.53	-0.50	1.52
Sigara distincta	0.92	0.53	-0.50	1.52
Sigara nigrolineata	0.92	0.53	-0.50	1.52
Sigara nimitata	0.92	0.53	-0.50	1.52
Enallagma cyathigerum	0.13	0.54	-0.18	-0.40
Coenagrionidae	-0.41	0.57	1.18	-0.10
Cyrnus insolutus	-0.36	0.57	-0.80	-1.02
Caenis horaria	-0.09	0.57	1.07	-0.37
Gyrinus caspius	1.14	0.58	1.28	0.31
Sialis fuliginosa	1.14	0.58	1.28	0.31
Stylodrilus heringianus	-0.19	0.60	0.94	-0.42
Sericostoma personatum	0.32	0.61	0.57	0.06
Limnius volckmari	-0.31	0.62	0.35	-0.23
Nemouridae	0.13	0.63	0.56	-1.08
Cyrnus flavidus	-0.13	0.63	-0.55	-1.31
Erpobdellidae	-0.49	0.64	-0.27	-1.11
Leptophlebia vespertina	0.02	0.65	-0.23	-0.91
Plectrocnemia geniculata	0.28	0.66	-0.43	-0.21
Cyrnus trimaculatus	0.18	0.66	0.41	-0.39
Mystacides azurea	0.26	0.67	0.90	-0.01
Tinodes waeneri	-0.41	0.68	-0.21	0.14
Pericoma	-0.10	0.70	1.88	-0.19
Nematoda	0.19	0.72	0.00	-0.57
Helobdella Stagnalis	0.31	0.72	0.20	-0.02
Stylaria lacustris	0.12	0.73	-0.09	-0.86
Corixidae	-0.27	0.78	0.07	-0.68
Mystacides longicornis	0.35	0.82	0.26	-0.78
Polycentropus kingi	0.35	0.82	0.26	-0.78
Sympetrum	0.35	0.82	0.26	-0.78
Leuctra sp.	0.64	0.86	0.61	0.20
Baetis rhodani	0.64	0.88	-0.43	-0.38
Ernodes articularis	0.64	0.88	-0.43	-0.38
Gammarus pulex	0.64	0.88	-0.43	-0.38
Tabanoidea	0.64	0.88	-0.43	-0.38
Ameletus inopinatus	-0.37	0.91	0.10	0.40
Rantus exsoletus	0.92	0.92	-0.18	0.15
Gyrinidae	0.28	0.92	0.09	-1.17
Elminthidae	0.43	1.01	-1.66	-0.83
Dreissenidae	-0.16	1.04	-0.21	-0.35
Leuctra fusca	-0.16	1.04	-0.21	-0.35
Sympetrum nigrescens	-0.16	1.04	-0.21	-0.35
Tabanidae	-0.16	1.04	-0.21	-0.35
Ephemeroptera	0.09	1.05	-0.17	0.35
Lumbriculus variegatus	0.92	1.11	-0.02	-0.54
Rantus bistriatus	0.92	1.11	-0.02	-0.54
Haliplus obliquus	0.29	1.19	-0.52	0.01
Oxyethira sp.	0.11	1.23	-0.34	0.15
Micropterna sp.	0.59	1.24	-0.22	-0.22
Baetidae	-0.11	1.30	-0.38	0.37
Isoperla grammatica	-0.06	1.49	-0.60	0.41

5.4.3 Species distributions and labile inorganic aluminium

The relationship between species distributions and Al_{lab} is of particular interest in WFD60 since Al_{lab} is known to be highly toxic to many aquatic animals, while its presence in toxic concentrations is highly indicative of acidification (see Section 3.3). Unfortunately Al_{lab} is not routinely measured by the UK environment agencies and we are therefore only able to examine relationships for this restricted dataset, enhanced by the inclusion of data for eight extra sites provided by the Fisheries Research Services Freshwater Laboratory, Pitlochry. Figure 5.3 provides some indication of a relationship between Al_{lab} concentration and species richness (as determined by the total number of species identified to species level). Of the nine sites with an annual average concentration of more than 25 µg l^{-1} none contain more than 10 identifiable species. Conversely, the seven sites with 14 or more species all have Al_{lab} concentrations of less than 20 µg l^{-1}. Clearly, however, low species richness is also common in a range of lakes with low Al_{lab} concentrations.

Figure 5.3 The relationship between labile inorganic aluminium concentration and the number of species identified to species level. Lines represent a fitted GAM model.



Relationships between individual taxa and Al_{lab} are provided in Figure 5.4, with respect to presence absence distributions and probability of occurrence (as modelled by the GAM curves). Several taxa provide an indication of acute Al_{lab} sensitivity. Unfortunately the size of the dataset is too restrictive to draw firm conclusions, but overall these plots are consistent with the hypothesis that Al_{lab} may exert a strong influence over species distributions in acidic lakes.

Figure 5.4 Presence/absence of the more common taxa in the interim dataset in relation to labile inorganic aluminium concentration. Curves illustrate GAM functions for presence/absence (i.e. probability of occurrence); dotted lines indicate 95% confidence intervals. Species names provided in Appendix 2

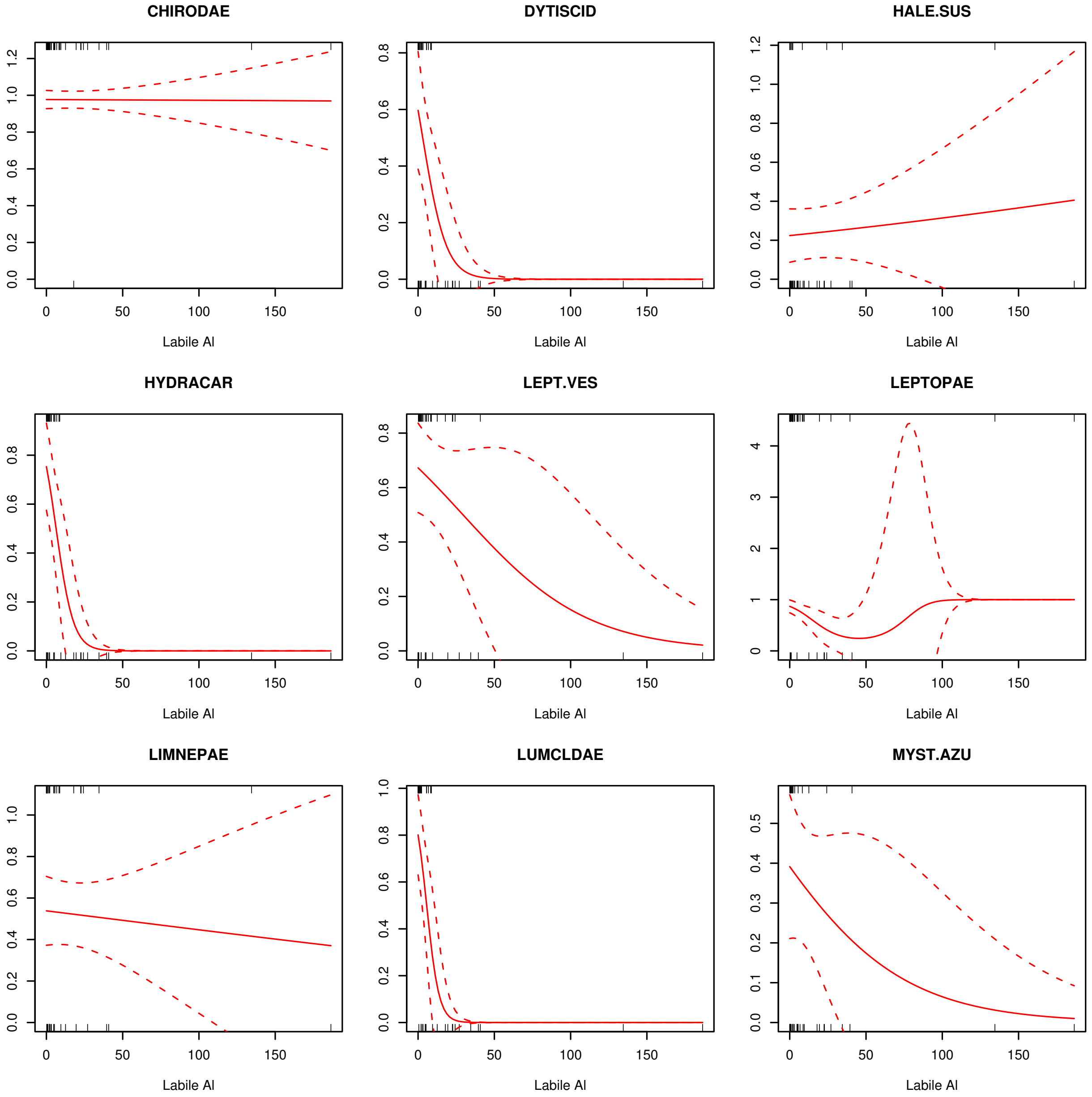


Figure 5.4 continued

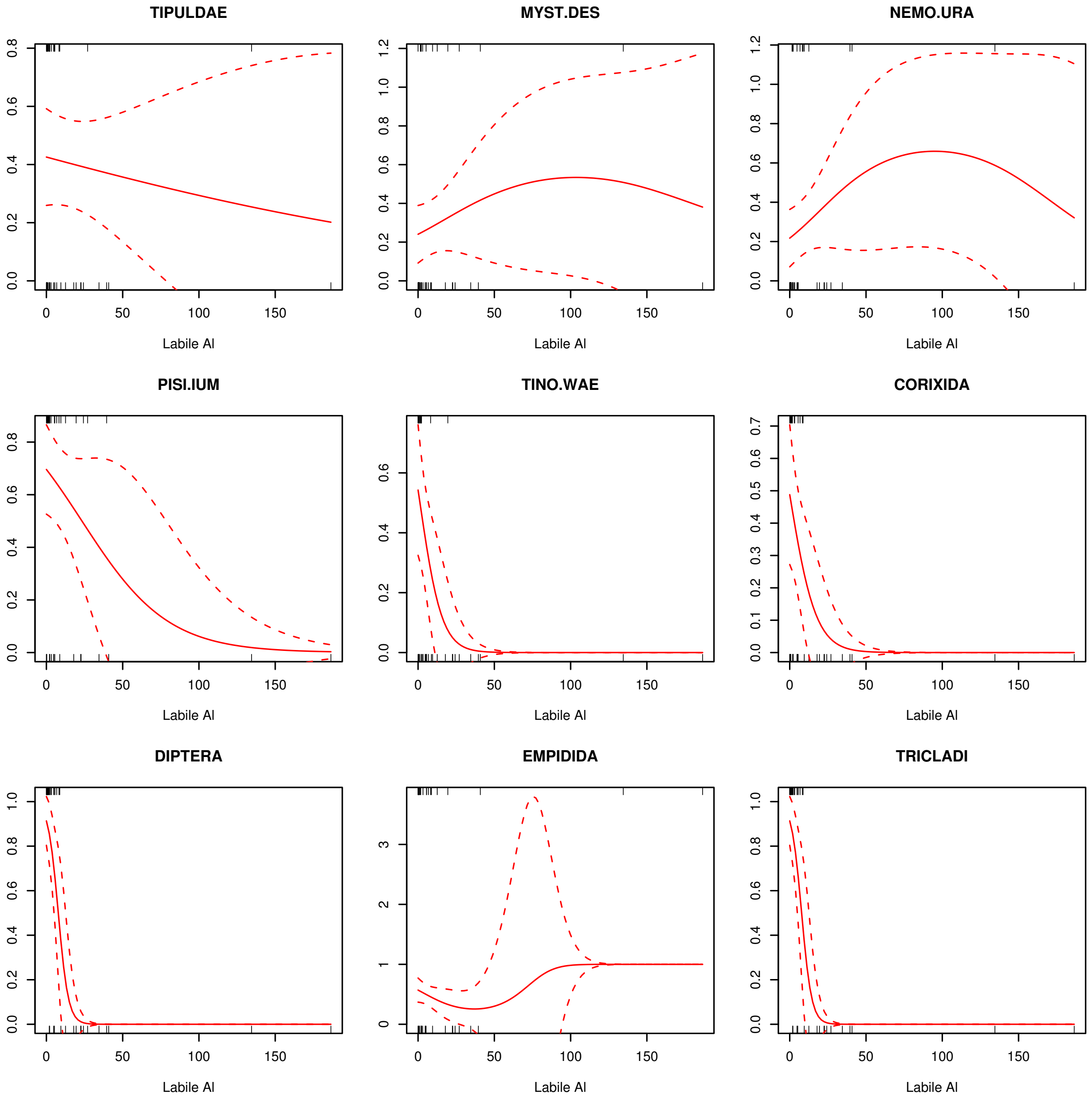
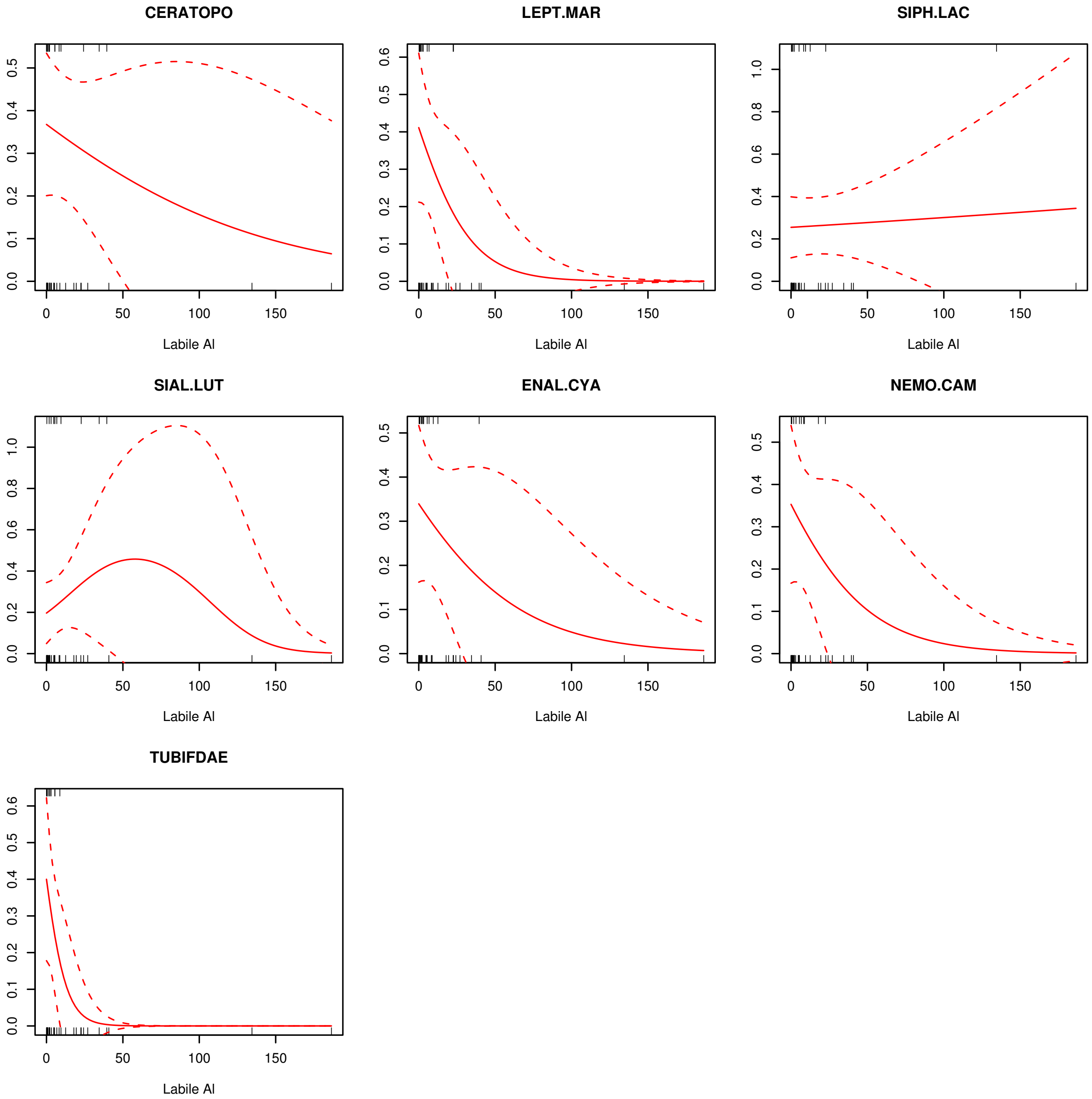


Figure 5.4 continued

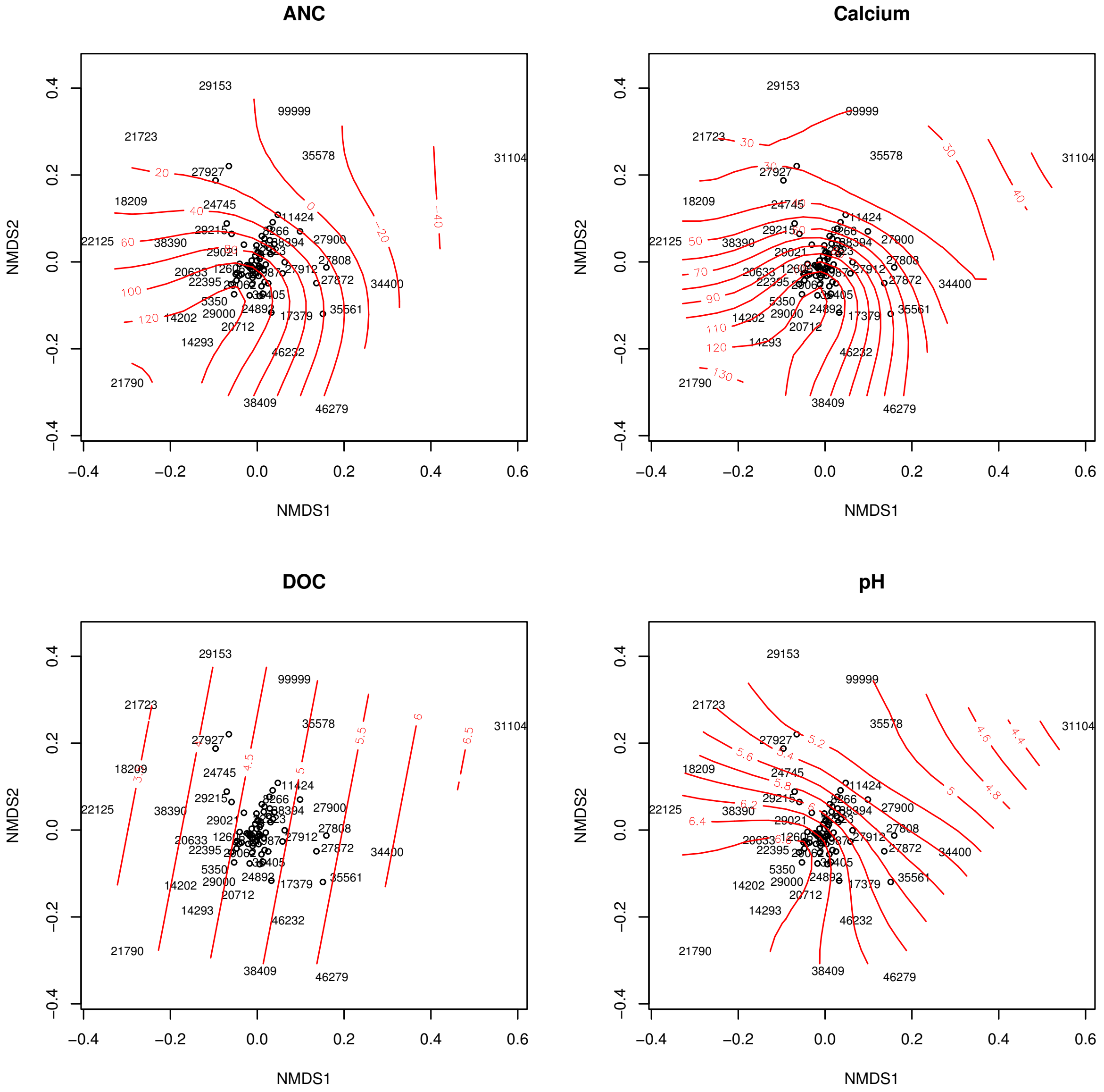


5.5 Exploratory Analysis of the full WFD60 dataset

5.5.1 Site ordination

Figure 5.5 is a non-metric multidimensional scaling (NMDS) ordination plot of the 105 sites in the WFD60 training set based on the species chord distances (i.e. dissimilarities). Overlain on these plots are fitted surfaces representing contours for gradients of ANC, Ca^{2+}, DOC and pH, based on an additive model which relates NMDS axes scores to these variables. These plots illustrate a generally satisfactory distribution of sites across the key acidity gradients and obvious relationships between species dissimilarity and acidity. However, the plots also emphasise the particularly poor coverage of more acidic sites.

Figure 5.5 Non-metric multidimensional scaling (NMDS) ordination plots of the 105 sites in the WFD60 database, based on macroinvertebrate species chord distances. Hence sites with the most similar species assemblages lie closest together. Contours represent gradients of ANC, Ca^{2+}, DOC and pH. Sites names are represented by WBID codes.



While sites with a mean pH below 6 are common, most sites in the dataset have an ANC above 60 µeq l^{-1}. Figure 5.6 is based on the same NMDS scaling but has macroinvertebrate weighted average site scores superimposed. The plots show that the most acidic sites are dominated by taxa such as the Hemipteran *Arctocorisa germari*, the Dytiscid beetle *Agabus arcticus* and the Corixid *Glaenocorisa propinqua*, while those at the other extreme are represented by the Dytiscid beetle *Oreodytes septentrionalis*, the caddis species *Agraylea multipunctata* and *Tinodes waeneri*. Unfortunately most taxa names are hidden from view in this plot due to size limitations.

5.5.2 Macroinvertebrate distributions on ANC and Ca^{2+} gradients

The presence/absence of taxa which occur in 10 or more sites (full dataset), across gradients of ANC, and Ca^{2+} (the two key variables for the WFD60 physico-chemical classification) are presented in Figures 5.7 and 5.8 respectively. The two plots show many similarities, which is not surprising given the strong covariance between ANC and Ca^{2+} in the full dataset. As in Figure 7.4 a number of taxa show very clear distributions. The majority of species show a rise in probability of occurrence with increasing ANC, but some, such as *Amphinemura sulicollis* (AMPH.SUL), *Oulimnius* sp. (OULI.IUS) and *Hydracarina* sp. ((HYDRACAR) appear to group toward the middle of the gradient and are found neither in very acid or high ANC waters. The stonefly *Nemoura cambrica* (NEMO.CAM) is one of very few species to be confined to the most acidic sites only.

Unsurprisingly, given the tendency for an increase in the probability of occurrence of individual taxa, species richness, as determined by the number of species identified to species level, also shows a very marked relationship with ANC and Ca^{2+} (Figures 5.9 – 5.10). Beneath an ANC of 40 µeq l^{-1} most sites contain no more than ten defined species, whereas above 60 µeq l^{-1} the vast majority of sites contain more than ten. Most sites with an ANC < 10 µeq l^{-1} contain six defined species or fewer. Whereas the relationship with ANC appears stepped, the relationship with Ca^{2+} is more linear, if more scattered, on most sections of the gradient.

Figure 5.6 Non-metric multidimensional scaling (NMDS) ordination plots of the 105 sites in the WFD60 database, based on macroinvertebrate species chord distances. Species positions represent a weighted average of the scores of the sites in which they occur.

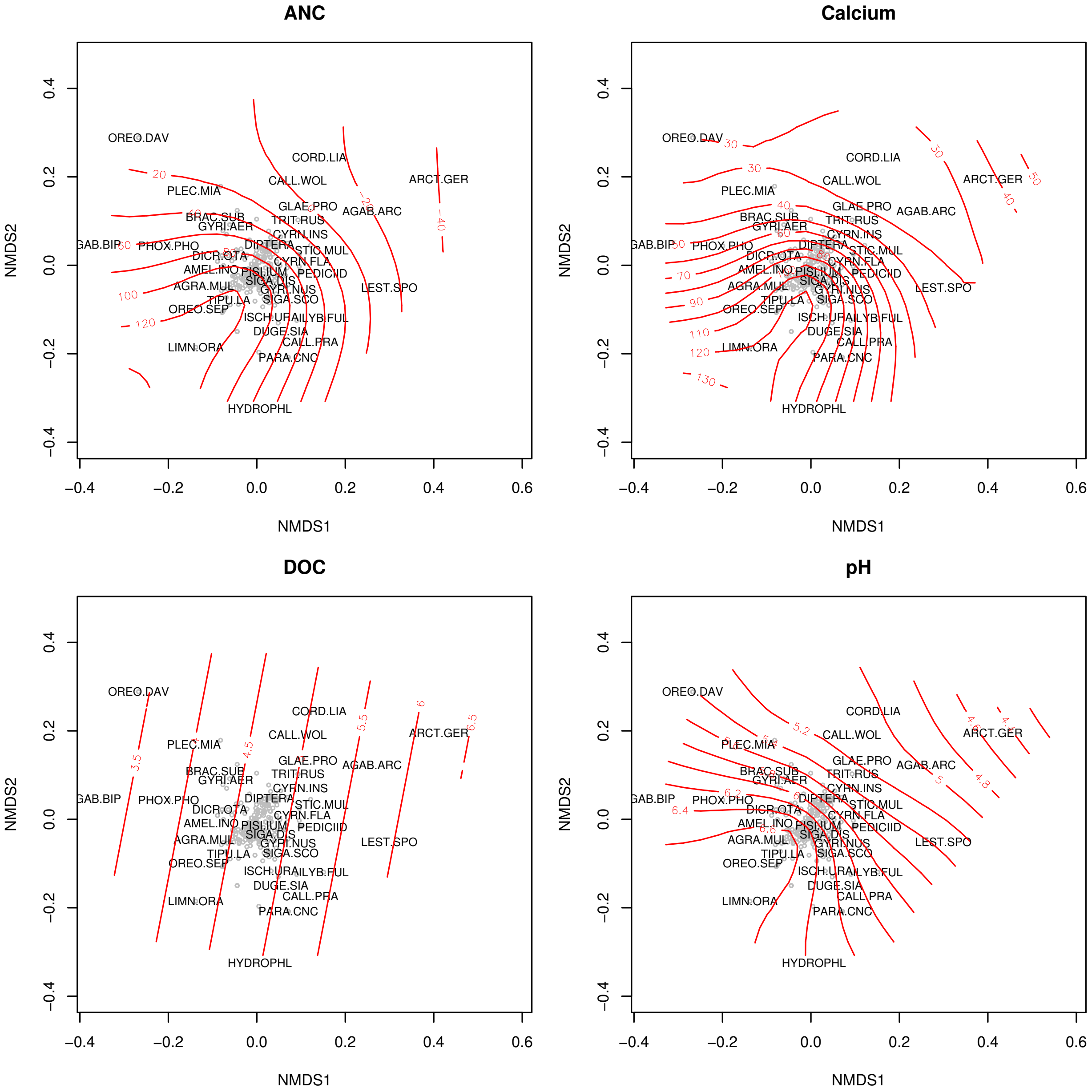


Figure 5.7 The presence/absence of taxa which occur in 10 or more sites (full dataset), across the ANC gradient (in µeq l^{-1}). Black line represents a GAM function (Poisson error distribution and logit function).

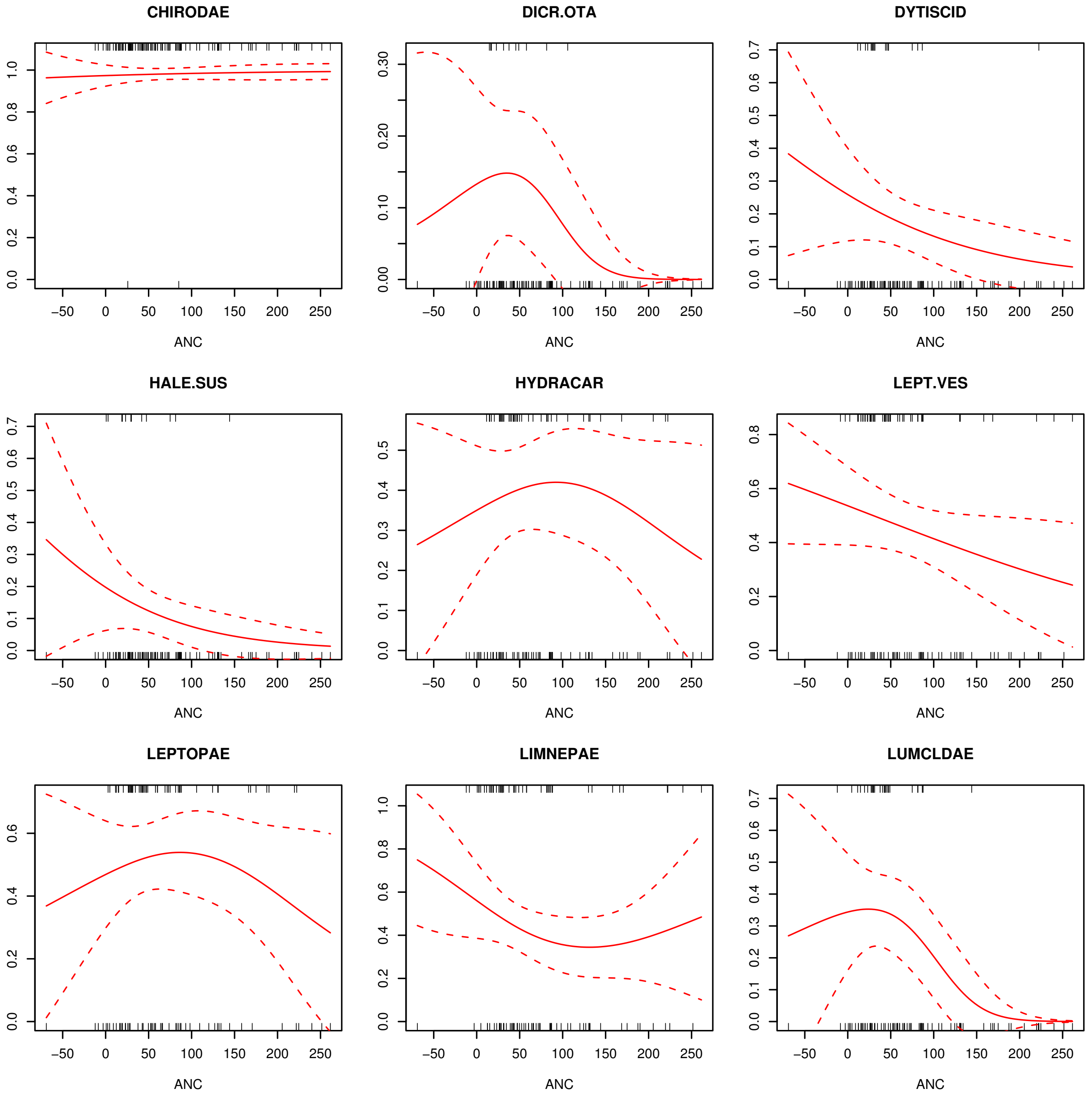


Figure 5.7 continued

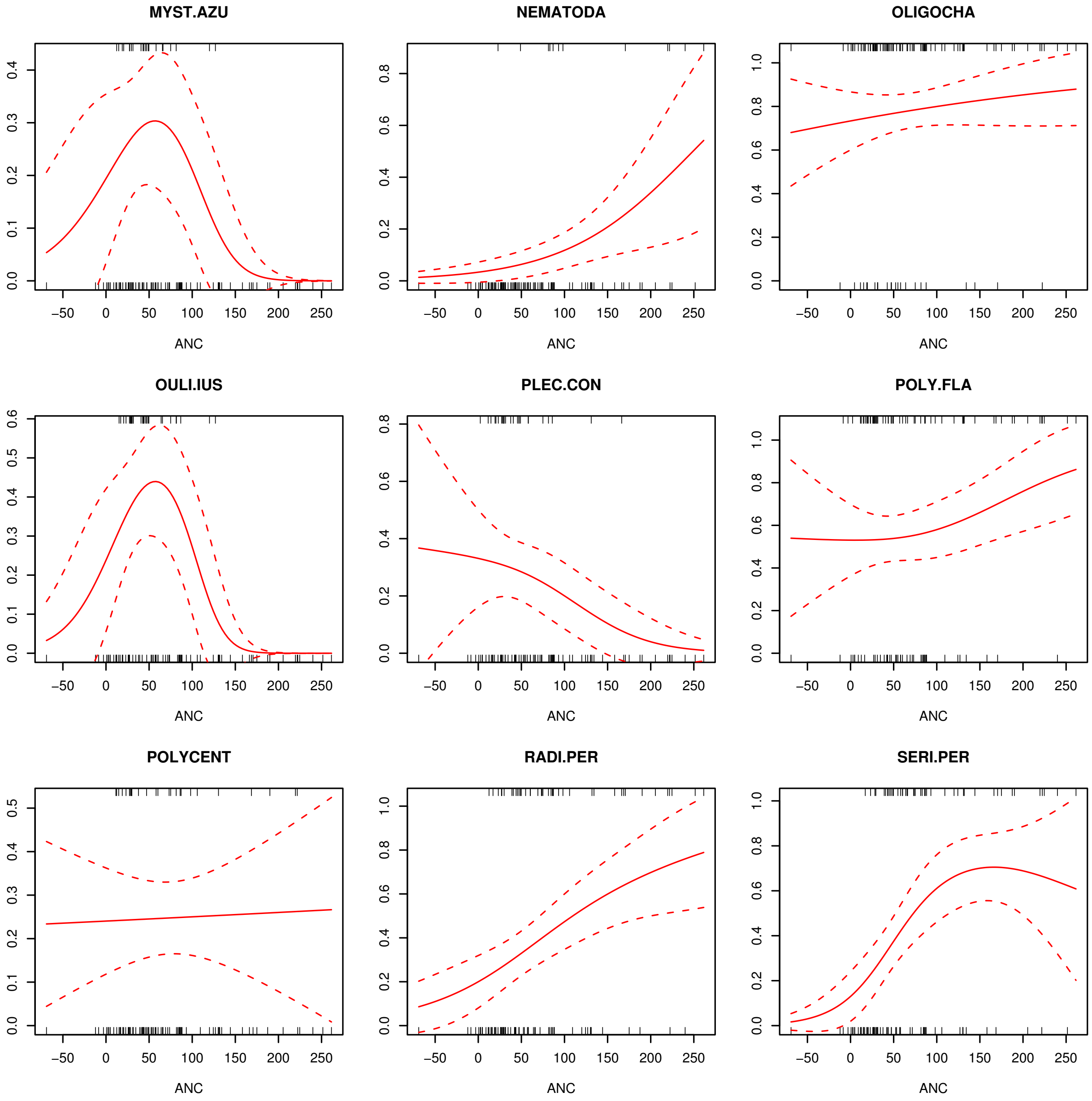


Figure 5.7 continued

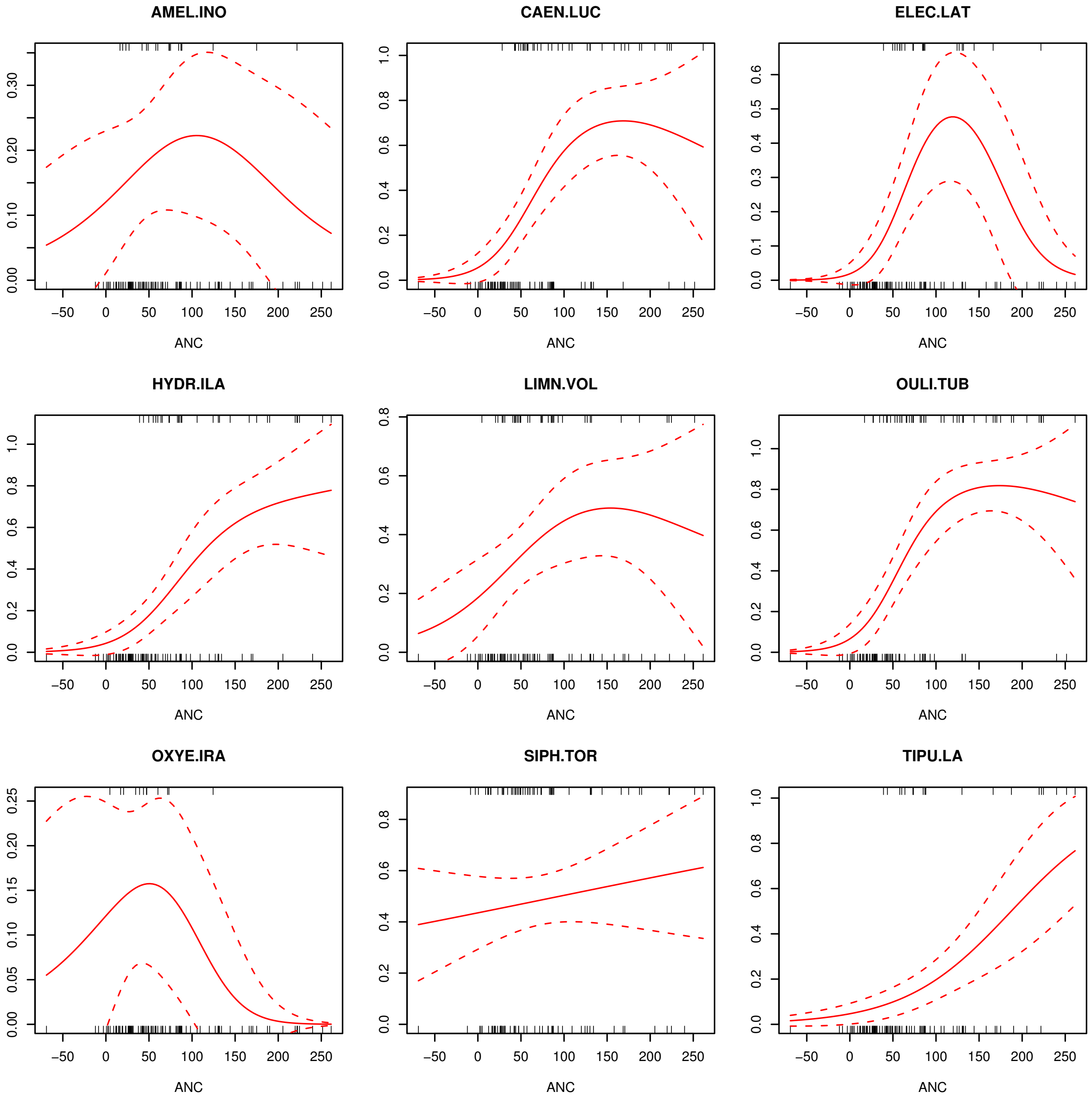


Figure 5.7 continued

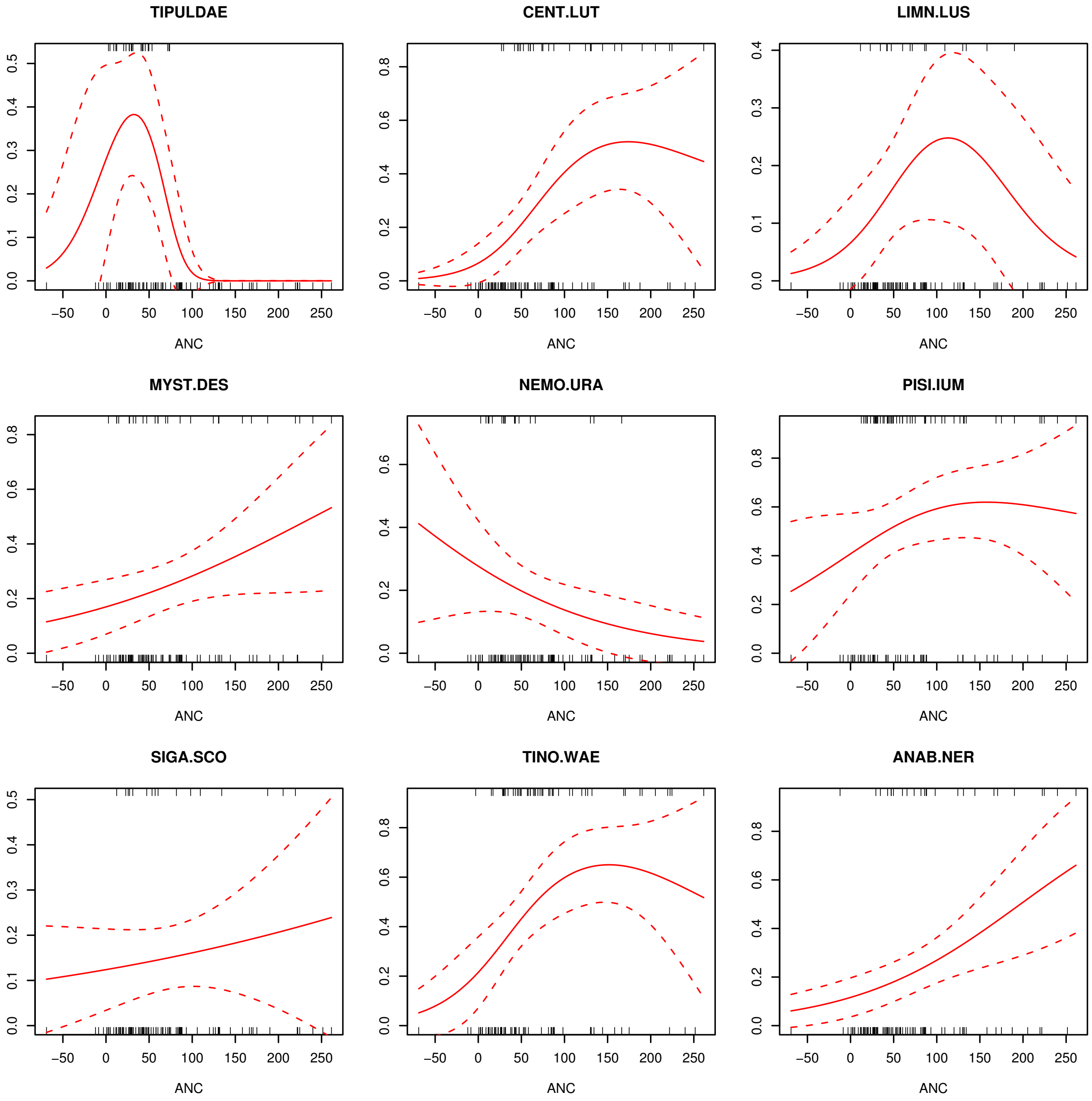


Figure 5.7 continued

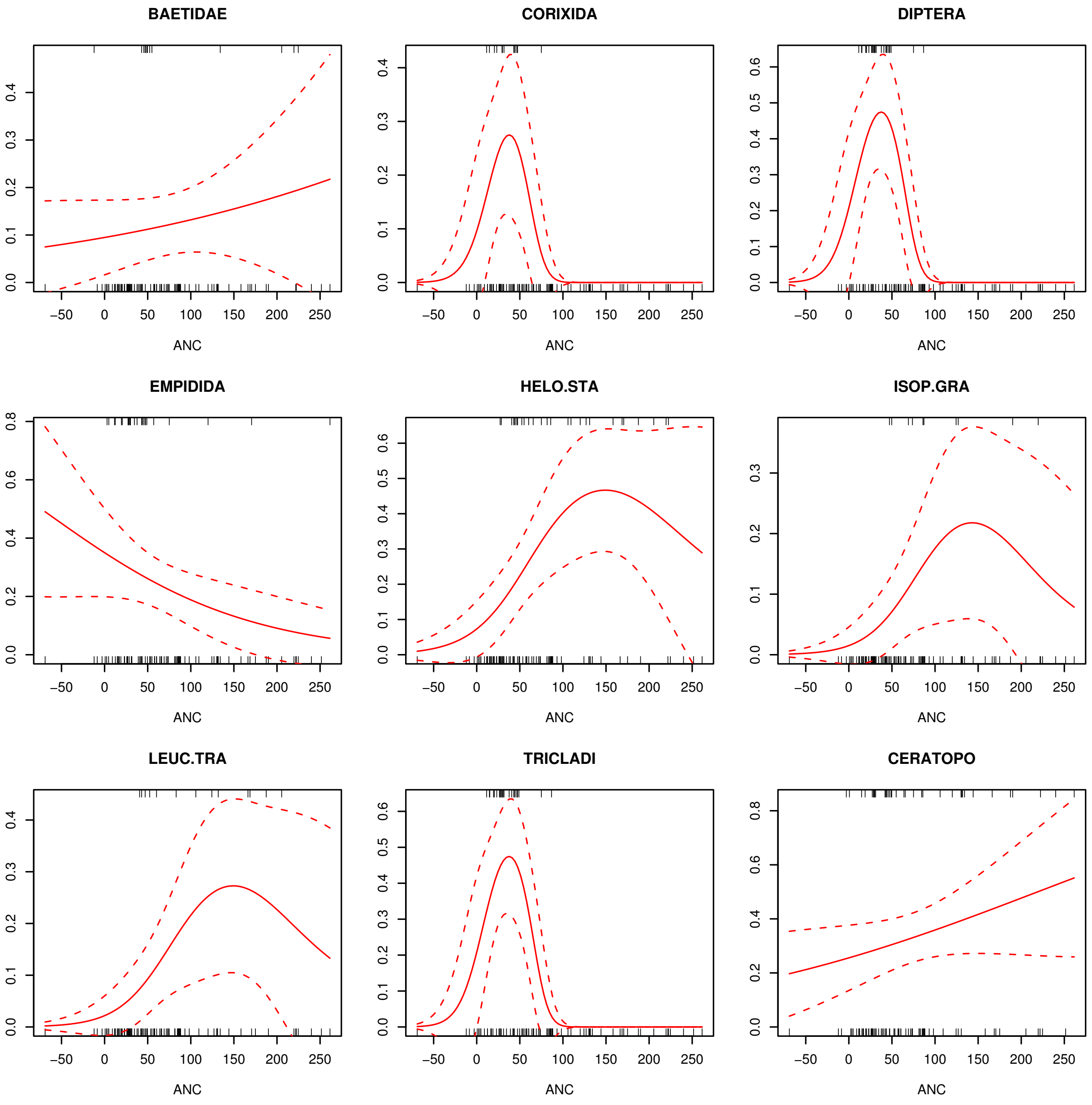


Figure 5.7 continued

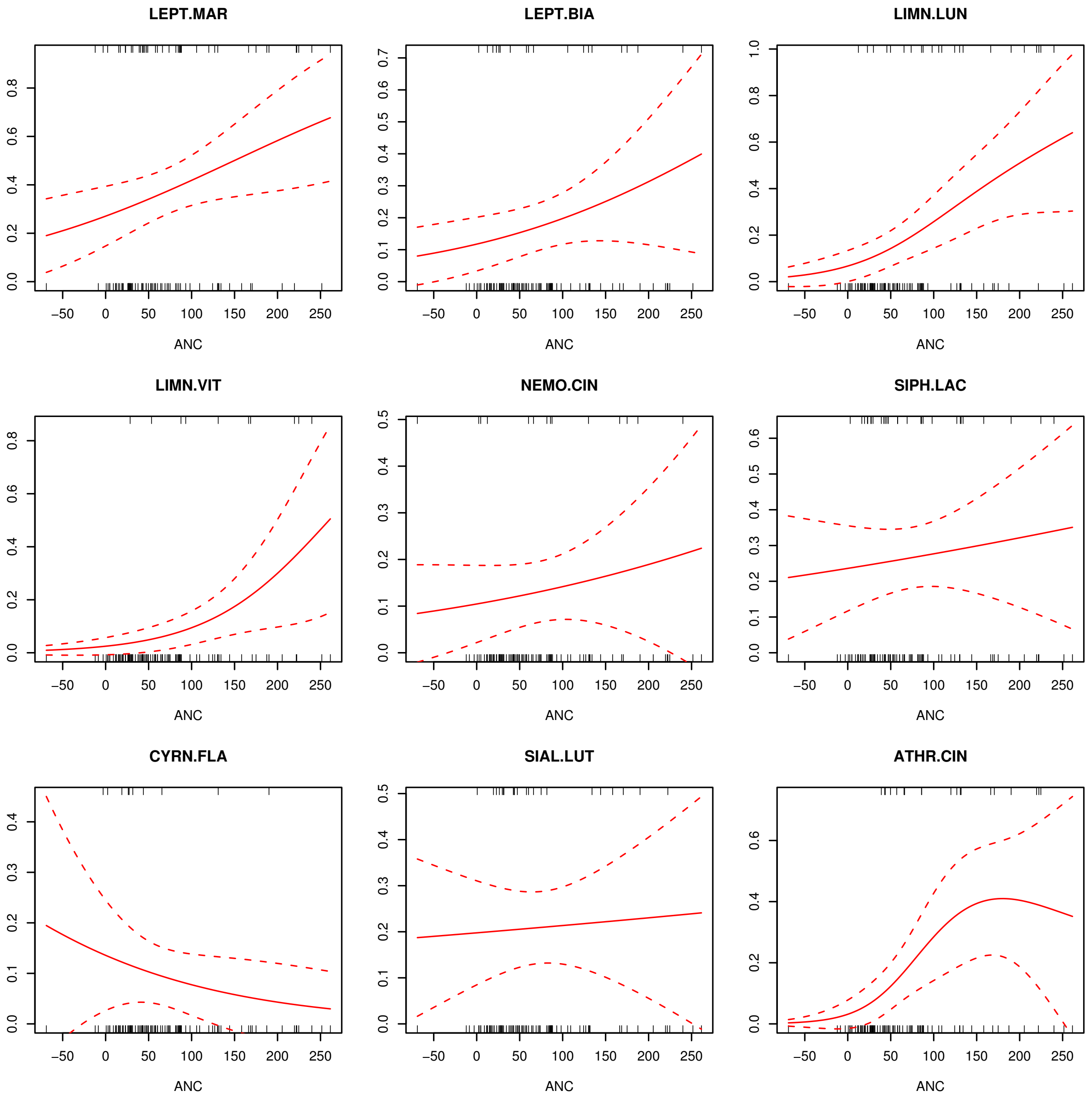


Figure 5.7 continued

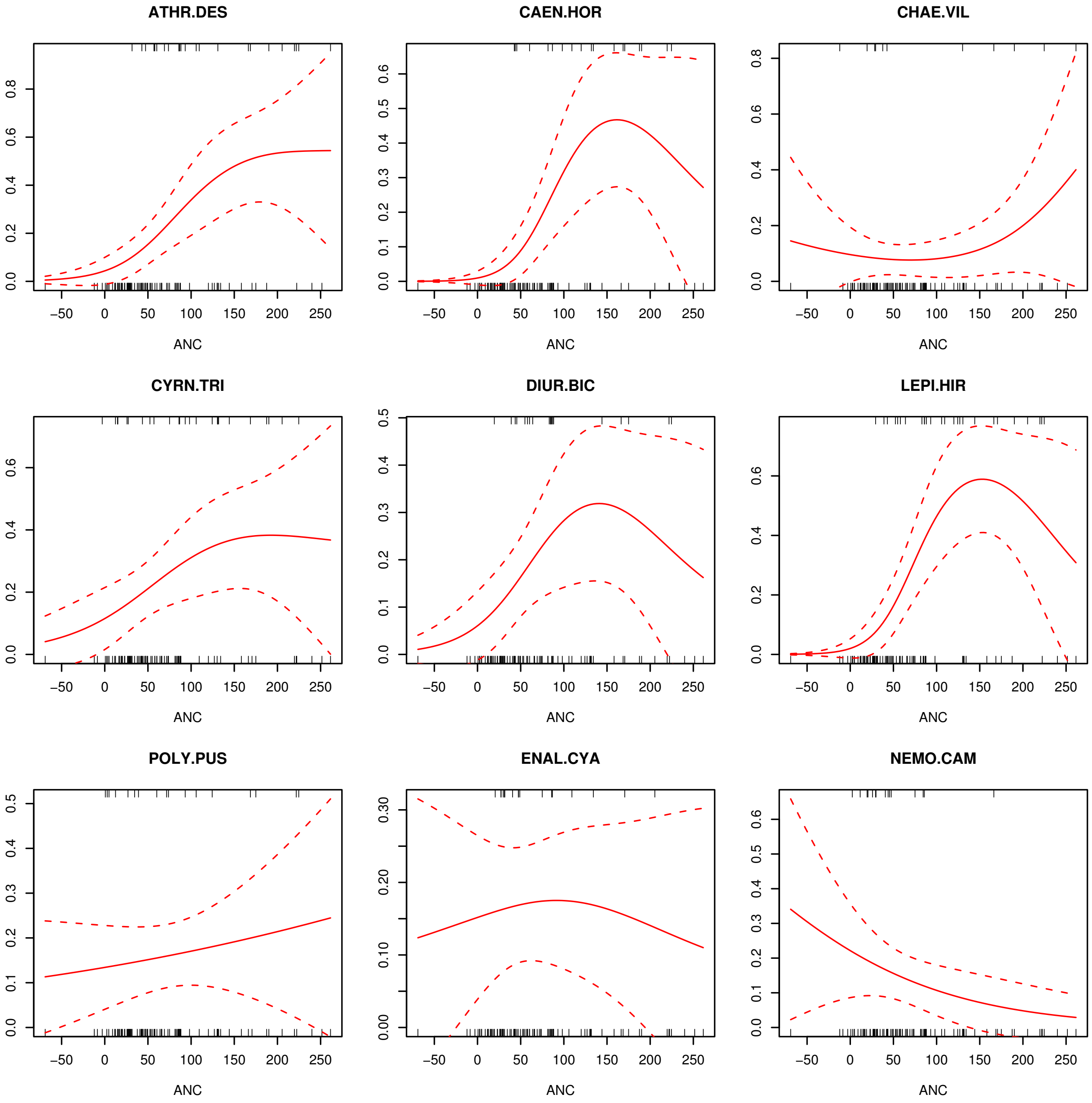


Figure 5.8 The presence/absence of taxa, which occur in 10 or more sites (full dataset), across the calcium gradient (in µeq l^{-1}). Black line represents a GAM function (Poisson error distribution and logit function).

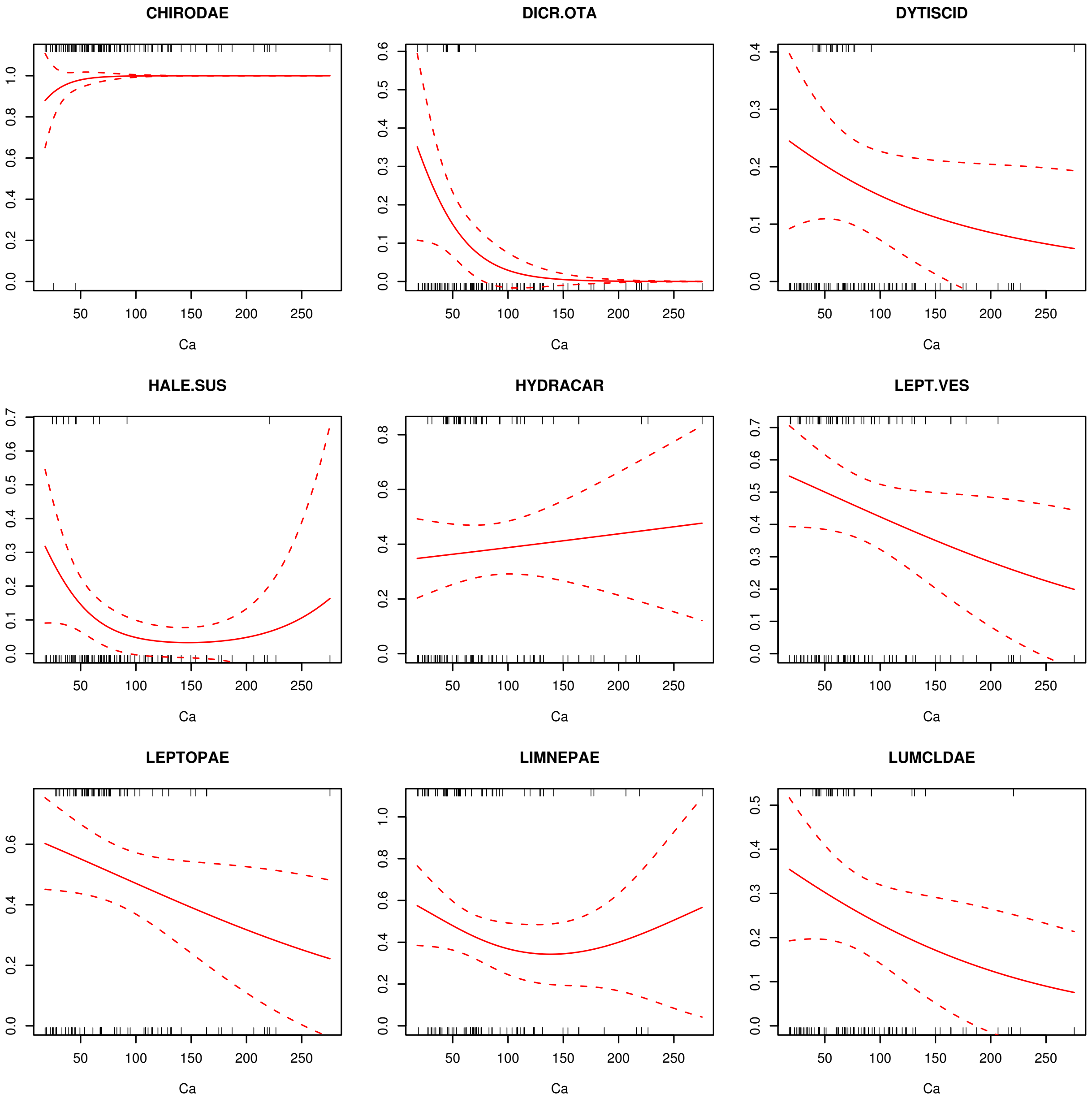


Figure 5.8 continued

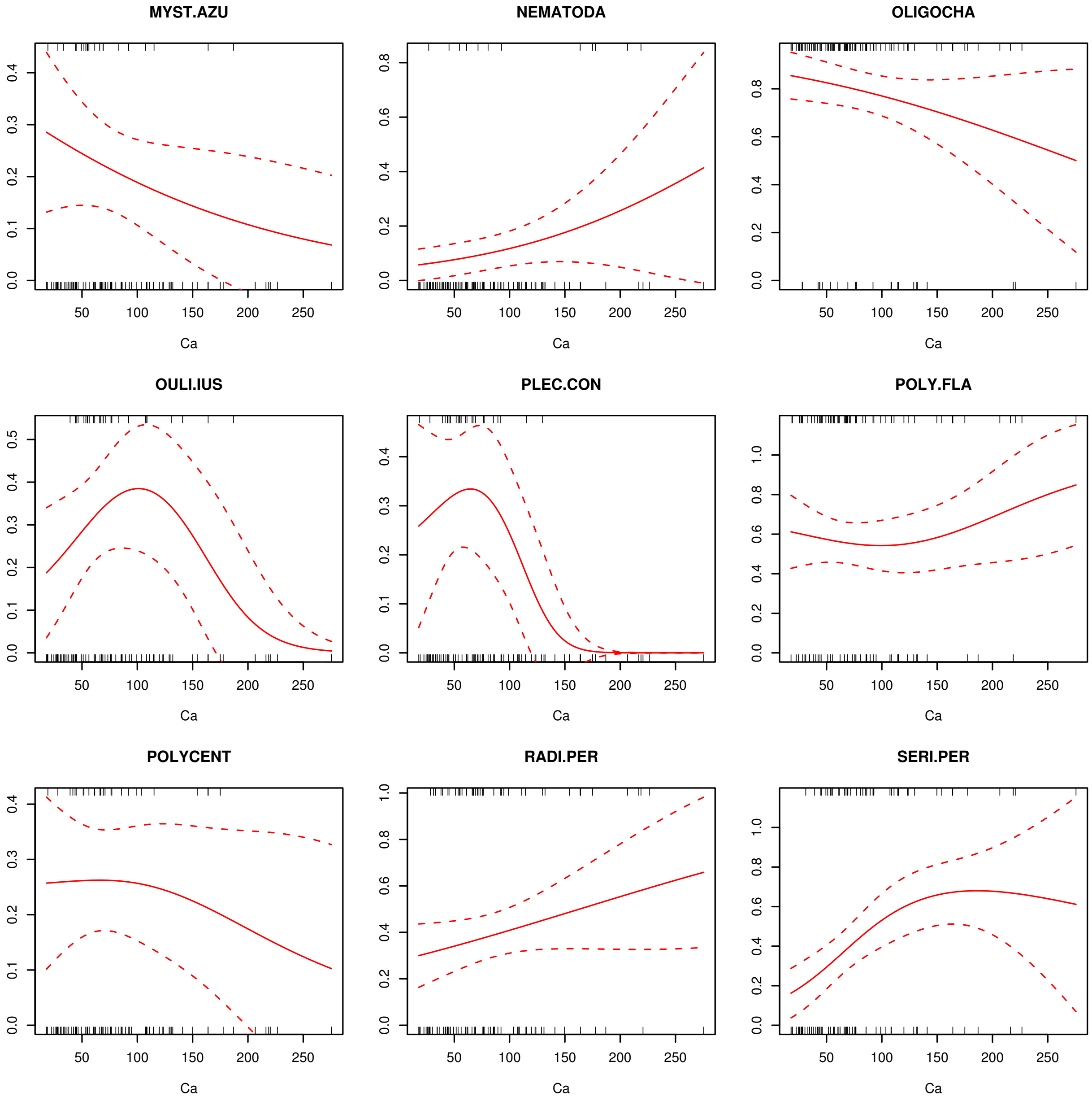


Figure 5.8 continued

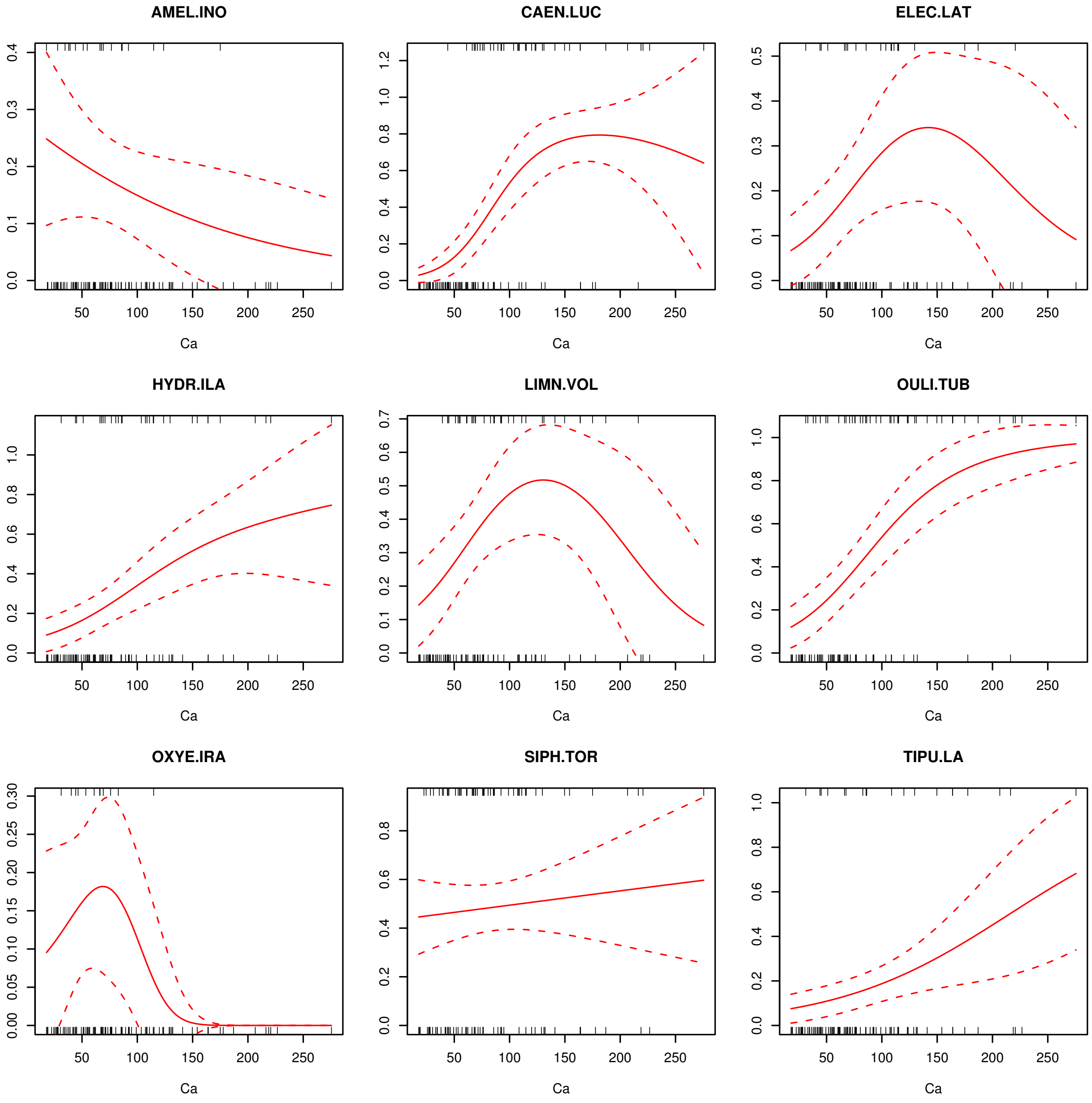


Figure 5.8 continued

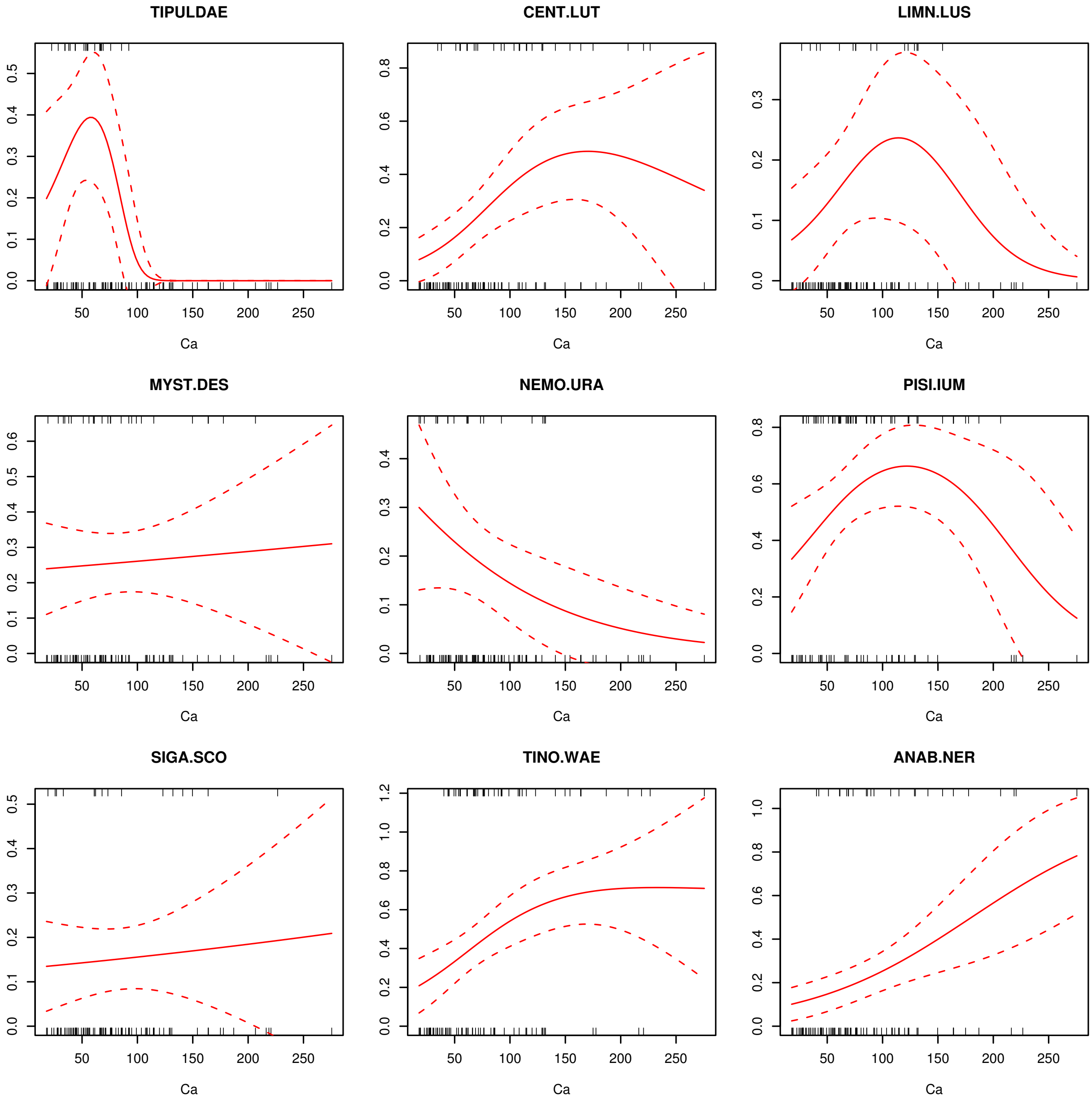


Figure 5.8 continued

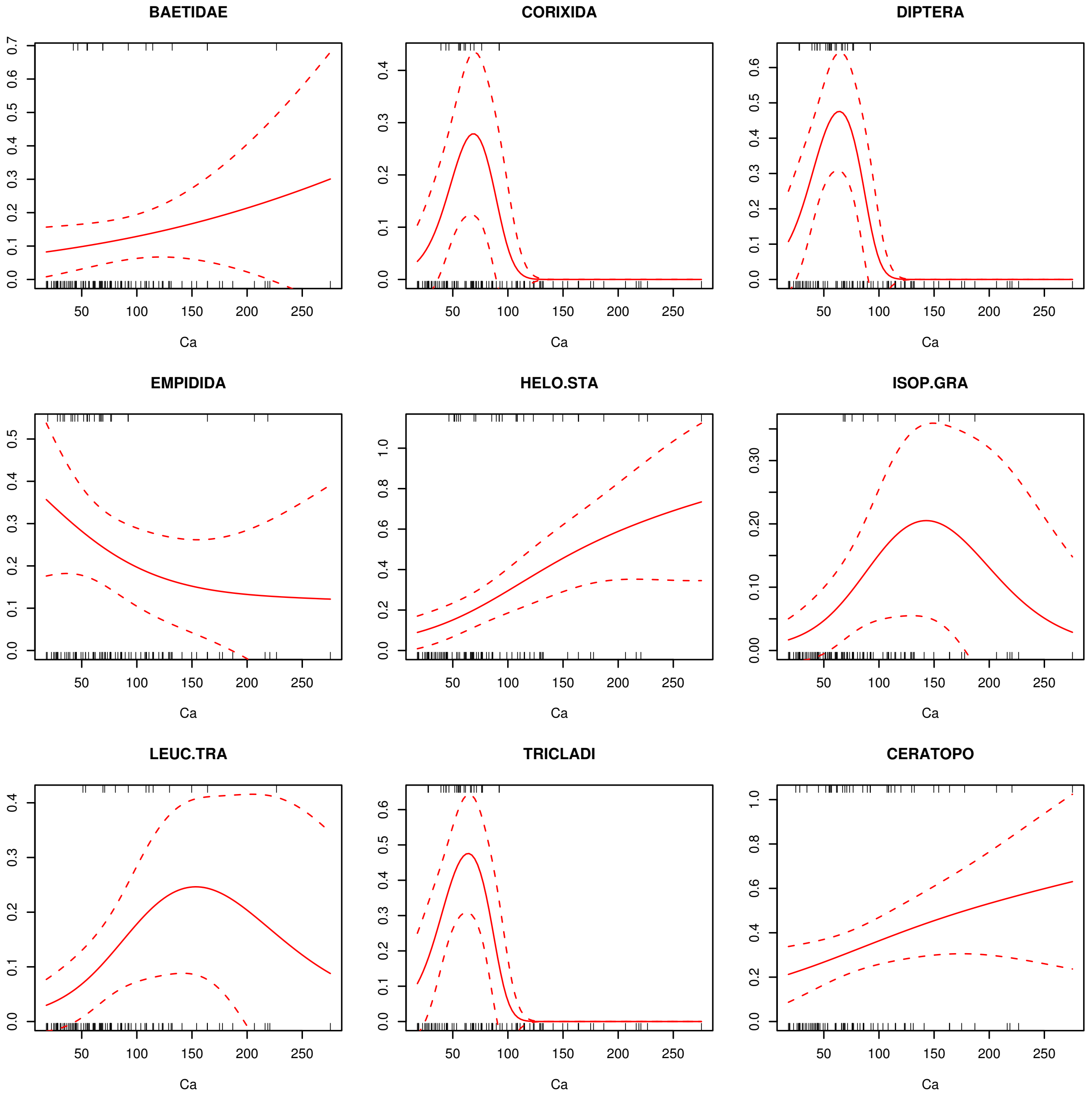


Figure 5.8 continued

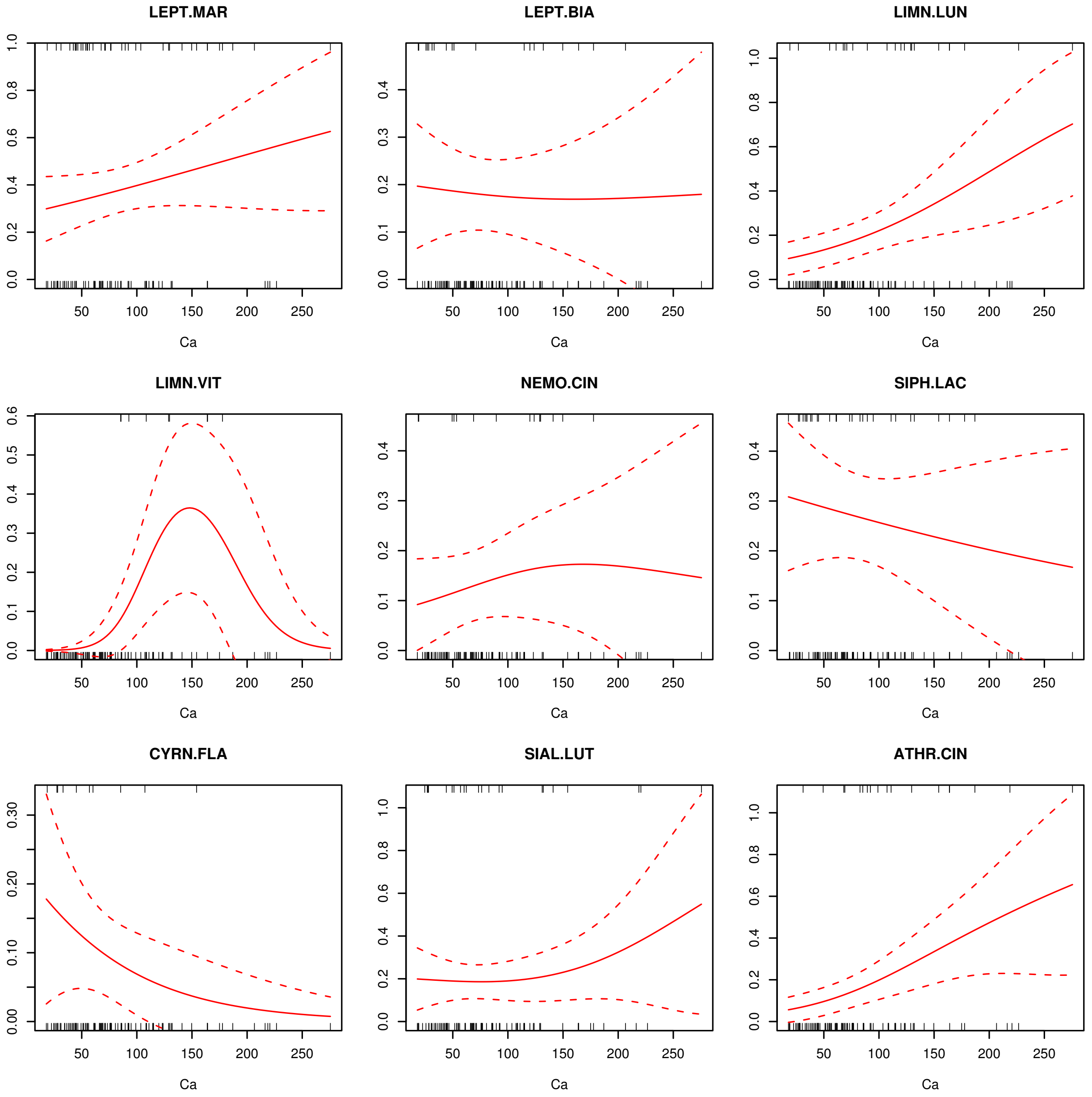


Figure 5.8 continued

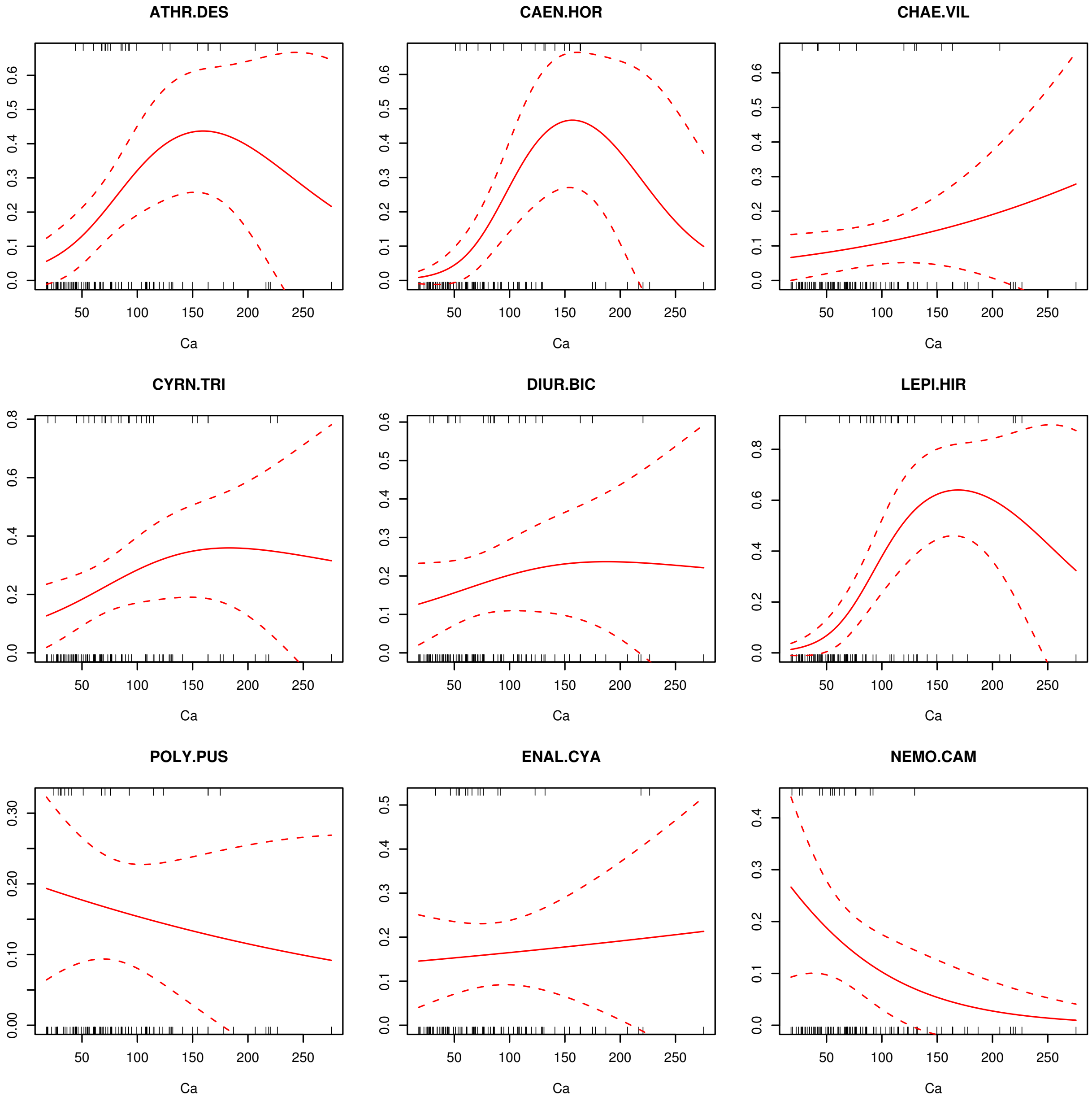


Figure 5.9 The number of taxa identified to species level related to mean ANC for the 105 sites in the WFD60 training set. Lines represent a GAM function (Poisson error distribution and log-link function.

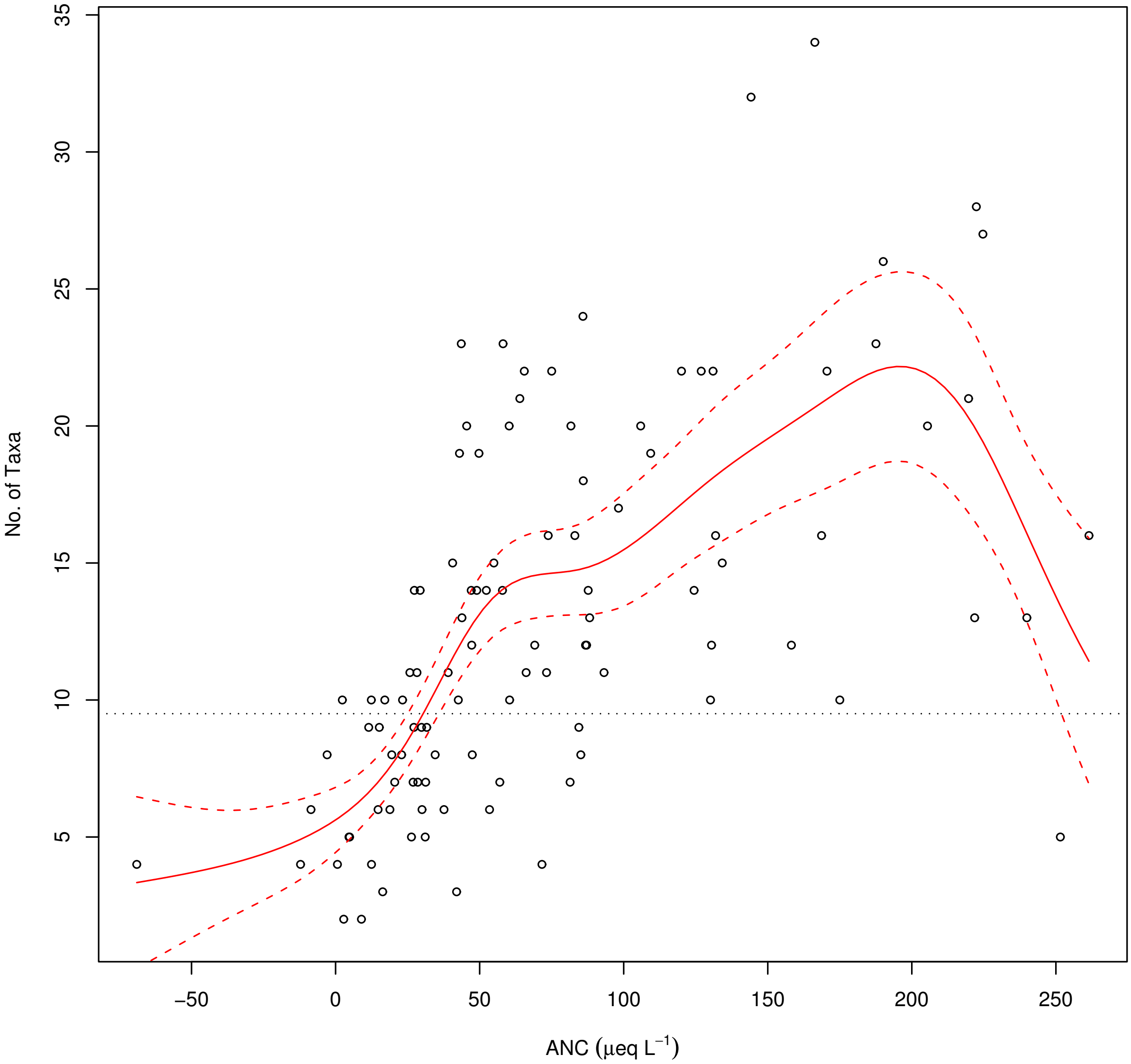
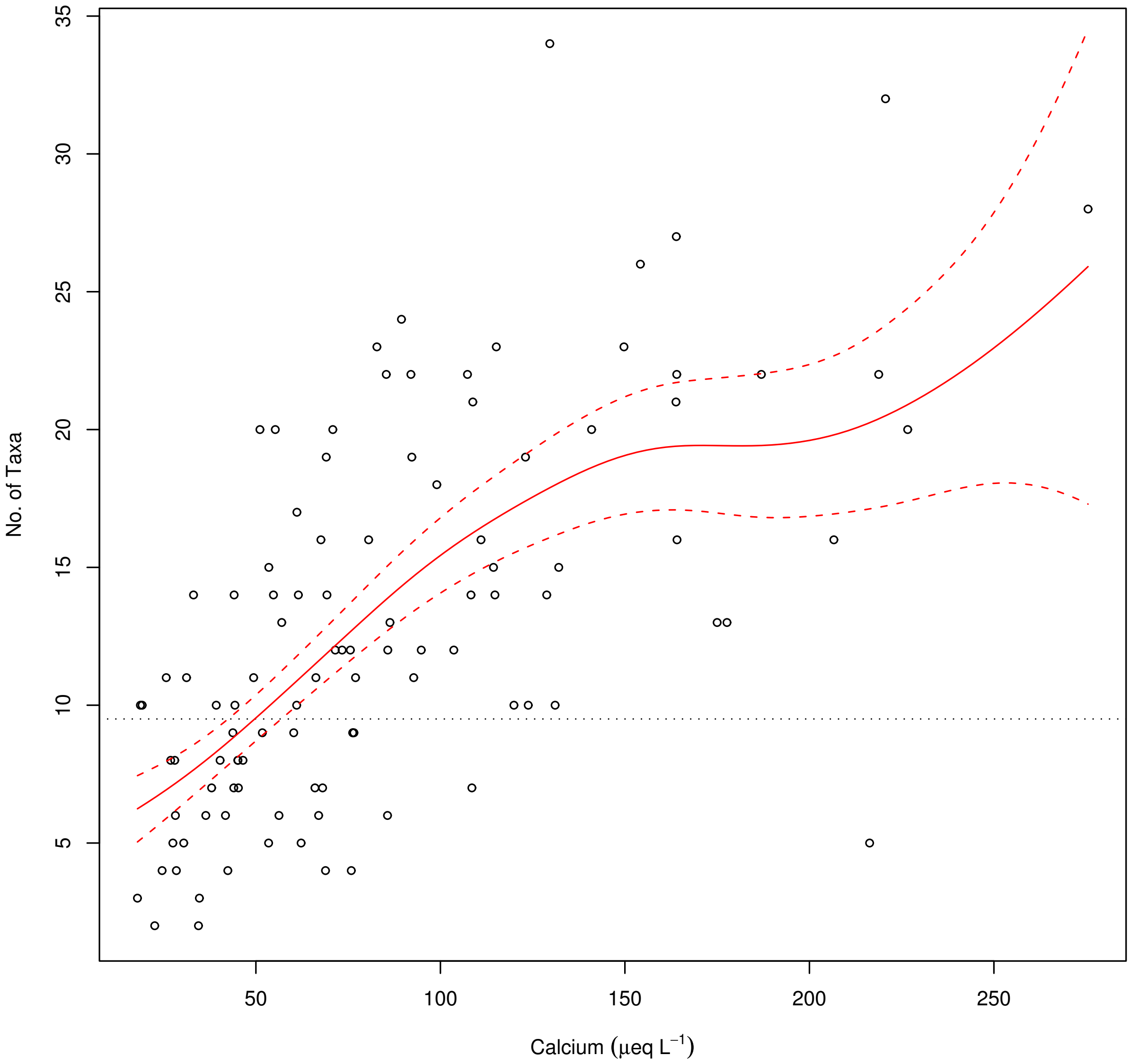


Figure 5.10 The number of taxa identified to species level related to mean Ca^{2+} for the 105 sites in the WFD60 training set. Lines represent a GAM function (Poisson error distribution and log-link function.



5.5.3 Summary of exploratory analysis

This analysis shows that macroinvertebrate structure can be tightly linked to ANC. The fact that the majority of taxa show an increasing probability of occurrence with rising ANC results in a very strong gradient in species richness. However this gradient shows a clear stepped distribution and is steepest between 0 – 60 µeq l^{-1}. The overall pattern in macroinvertebrate species diversity indicates that “High” biological status (according to WFD normative definitions) might be the norm for sites with an ANC greater than 60 µeq l^{-1}, whereas the abrupt reduction in diversity as ANC approaches zero suggests that the fauna of sites with an ANC of less than zero will be in a condition that could be described as between “Poor” and “Bad”.

6 WFD TOOL DESIGN PRINCIPLES

6.1 Other WFD Schemes under development

Several WFD classification tools are currently being developed in the UK under the supervision of UKTAG. The design methodology for most of these is similar and involves four main steps.

1) Reference sites (i.e. a sub-set of those assumed to be of High status) are identified for each typology, using "expert judgment".
2) Biological assemblages in a "training set", including those for reference sites, are related to a physico-chemical pressure gradient, such as phosphorus concentration, using multivariate ordination methods such as canonical correspondence analysis (CCA).
3) Sample scores derived from the ordination procedure are divided by sample scores for reference lakes within the same typology to provide an ecological quality ratio or EQR for each lake.
4) EQRs are then related to biological normative definitions, such as the relationship between stress tolerant and intolerant species, and this is used to divide up the gradient into the five WFD classes introduced in Section 1.

Class membership is then subjected to uncertainty analysis to ascertain the likelihood that a biological sample will be allocated the appropriate damage class, given the known susceptibility of the sample data to variability in sampling effort, time, space, etc..

Thus EQR derivation follows WFD guidance outlined in Annex 5 in a very literary manner, i.e.:

"..the results …..shall be expressed as ecological quality ratios for the purposes of classification of ecological status. These ratios shall represent the relationship between the values of the biological parameters observed for a given body of surface water and the values for these parameters in the reference conditions applicable to that body."

However, there is no explicit requirement in the Directive that an EQR must be calculated mathematically by dividing sample scores in the way outlined above. We argue that this clause may alternatively be interpreted as a qualitative requirement that the EQR must be based on comparisons of biological condition of a site with what is considered to be reference condition. If certain biological characteristics of high status can be considered universal, then any deviation from these characteristics may be used to derive an EQR score.

We argue that the commonly adopted procedure is prone to *a priori* uncertainties which are not subjected to rigorous analysis. First, no two lake ecosystems are identical now or in the past, however WFD "reference lakes" tend to be few. Uncertainties arise immediately, therefore, with regard to representativity of these lakes and hence the relative level of damage for lakes within the same typology for which the same reference condition is used. While a continuous EQR score is generated there are no grounds to believe a lake which has been allocated an EQR of, for example, 0.5 is less damaged than one with an EQR of 0.4, even within the same typology. The apparent continuity of the score, therefore, has limited ecological merit with respect to damage assessment.

Second, the procedure by which the EQR gradient is divided into damage classes is often based on subjective criteria such as the cross-over point between two biological indicator classes (themselves defined by relating to "pressure gradients" used in previous steps), rather than a mechanistic understanding of how the pressure is likely to influence the biological community.

Finally, this cross-over point, whilst being the optimal discriminator between the two groups of biological indicators, may not be an optimal decision threshold for discriminating between site damage classes. Again, the use of the cross-over point to fix the Good-Moderate boundary is not required by Annex V of the Directive and we argue that the widespread use of this criterion for setting this important boundary results from an overly prescriptive adoption of statements in the WFD. The cross-over point is used solely to describe what the Commission meant by "moderate" change over reference, not that this will necessarily be adopted as part of member states classification schemes. Overall, therefore, we feel this process is self-referential and of restricted ecological validity.

6.2 Classification under WFD60

We have proposed an alternative approach to lake classification to that discussed above. This is based on the following observations outlined in previous sections of this report:

a) In contrast to other pressures of concern to the WFD, lakes of "reference condition" may be particularly difficult to identify within the same biogeographic region; the most appropriate "reference conditions" for acidified lakes in north Wales, the Pennines and the English Lake District, may only be found in unacidified parts of the far north-west of Scotland, but these may not be sufficiently analogous to lakes much further south for climatic and geological reasons;
b) unlike other pressures of concern to the WFD the pressure of acidification can be predicted from current physico-chemistry;
c) high status, according to physico-chemical normative definitions, can be identified with some confidence on the basis of ANC;
d) a physico-chemical "good-moderate" boundary may be defined according to our understanding of the importance of Ca^{2+} in determining the likely ANC threshold for biological damage through acidification (based on physico-chemical and palaeoecological models); this threshold can be considered to be the point at which biological communities begin to differ "moderately" from reference and where major taxonomic groups are first likely to disappear, according to WFD normative definitions;
e) "poor" to "bad" status may also be defined on the basis of biological toxicity thresholds for aluminium, and relationships between Al_{lab} and ANC our data demonstrate that many species and taxonomic groups typical of acid sensitive lakes are excluded from lakes with an ANC below zero and the dominant reason for this is likely to be due to the coincidence of this threshold with substantially elevated levels of Al_{lab} in addition to low pH.

We are confident, therefore, that we can classify lakes in our training set using physico-chemistry in a manner that accords with biological normative definitions and evidence of the degree of departure of biological communities from reference state. In this report we go on to show how such classes can be predicted by the macroinvertebrate community, and how this can then be used as the basis for the WFD60 tool.

6.3 WFD60 proposed classification approach

Rather than generate EQRs for individual sites using assumptions on appropriate reference sites, and then attempt to divide the EQR gradient according to normative definitions into the five WFD classes, we set out to test whether it was possible to use our understanding of the relationship between physico-chemical indicators of damage and aquatic biology to derive *a priori* EQR compatible classes for sites, and then use a classification approach to predict membership of a class based on the macroinvertebrate characteristics of each site.

For example, according to our preliminary analysis of the data and information available in the scientific literature, it is reasonable to assume that the macroinvertebrate community of sites with an annual mean ANC of >60 µeq l-1 are unlikely to differ significantly from reference condition (with respect to acidification). Providing there are no other major environmental constraints: taxonomic composition should correspond totally or nearly totally to unacidified conditions; there should be no sign of alteration in the ratio of sensitive to insensitive taxa; and, there should be no sign of any reduction in diversity from that found in acidified sites. The EQR of such a site should approach a value of 1, and could, for the sake of convenience, be allocated a score of 0.9.

Conversely, the macroinvertebrate community of any site with an ANC of < -50 µeq l-1, is highly likely to exhibit very low pH and highly toxic concentrations of Al_{lab}. Such a site is highly likely to support a very limited number of highly acid-tolerant taxa only and will deviate profoundly from reference condition with respect to taxonomic composition, abundance, ratios of sensitive to insensitive taxa and diversity. Its EQR must therefore approach zero and could again, for convenience, be allocated a score of 0.1. If we can derive classification rules which can determine the likelihood of a biological assemblage falling into these classes then we have the basis for a robust classification system which is compatible with WFD requirements.

6.3.1 Generation of a "damage matrix"

We used our understanding of the relationships between current ANC and calcium concentrations, and Al_{lab} concentrations, predictions of the extent of acidification, and our understanding of critical biological thresholds, to derive a damage matrix with which any site could be classified. This is presented in Table 6.1. Each of the 107 sites in the WFD60 database was therefore assigned a class according to these categories.

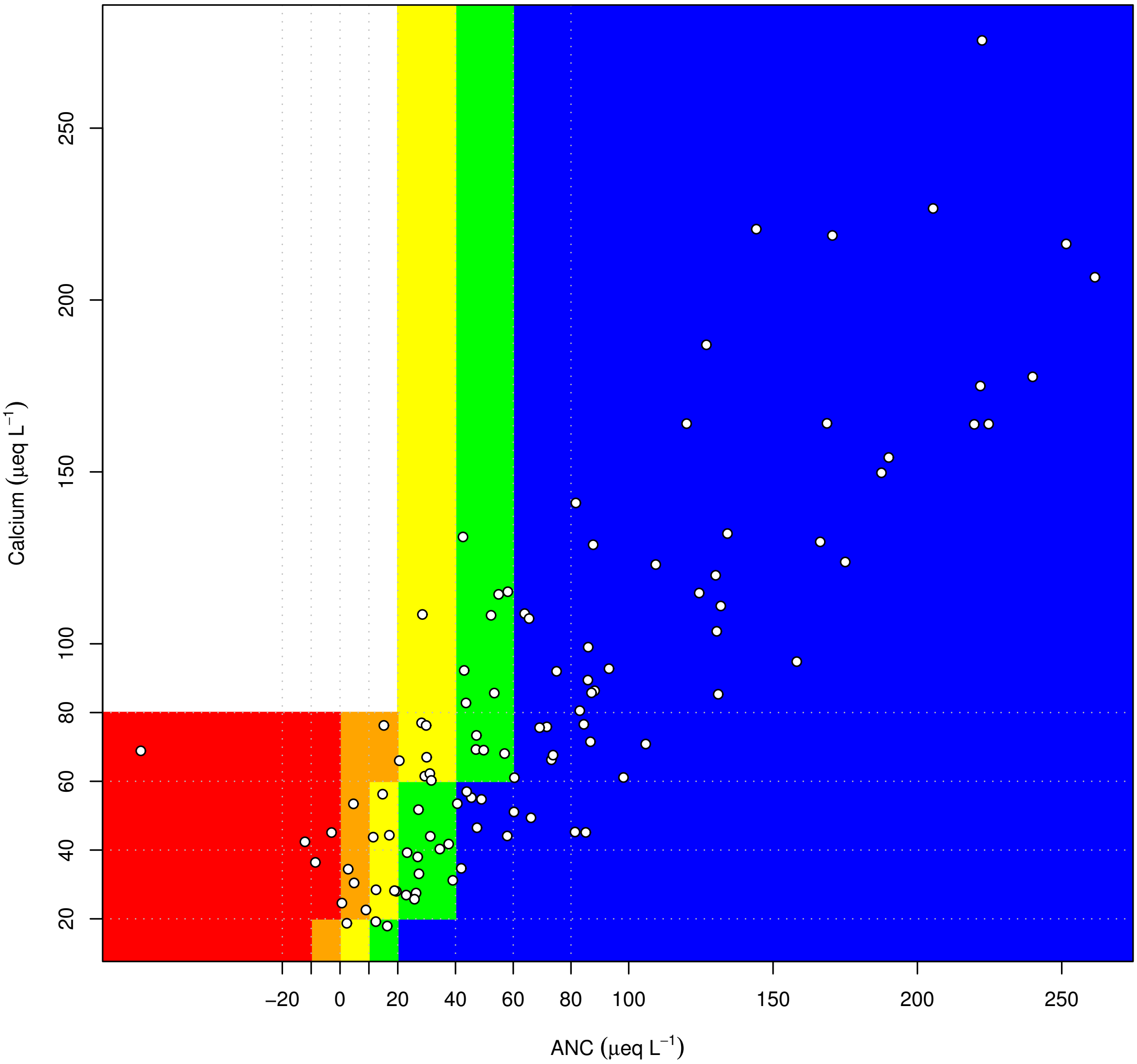
Table 6.1. Damage matrix, based on understanding of relationships between ANC and calcium concentrations and evidence from palaeoecological and hydrochemical models of acidification, and contemporary relationships with Al_{lab} and macroinvertebrate assemblage characteristics. Letters represent expert judgement on likely ecological status with respect to damage from acidification. H = High, G = Good, M = Moderate, P = Poor and B = Bad.

Ca^{2+} (µeq/l)	ANC group (µeq/l)								
	100-80	**80-60**	**60-40**	**40-20**	**20-10**	**10-0**	**0-(-10)**	**-10-(-20)**	**-20-(-40)**
0-20	H	H	H	H	G	M	P	B	B
20-40	H	H	H	G	M	P	B	B	B
40-60	H	H	H	G	M	P	B	B	B
60-80	H	H	H	M					
80-100	H	H	G	M					

Following preliminary data analysis, the paucity of samples in classes "Poor" or "Bad" resulted in modelling problems. The training data set was heavily biased to classes "High" and "Good" (50% and 30% of samples respectively). Moderate sites comprised just over 11% of the samples with the remaining c. 9% of samples in Poor or Bad status. This is illustrated in Figure 6.1, where there a very few samples in the areas coloured red (Bad), orange (Poor) or yellow (Moderate).

To work around this problem, classes "Poor" and "Bad" were merged into one "Poor-Bad" class. Also, as a result of our relative lack of confidence we had in defining the "High-Good" boundary it was also decided to merge these two classes. Consequently our classification would be based on fitting to only three status classes.

Figure 6.1 Distribution of sites according to ANC and Ca^{2+} concentration. Diagram shaded according to the damage matrix (Table 6.1).



6.3.2 Macroinvertebrate input data

Macroinvertebrate data were processed for subsequent analysis in 6.3.3 as follows:
Total abundances were first calculated for each Mixed Taxon Level taxa for each sample date for each site. The bulked data thus represented differing sample sizes depending on the origin of the samples. In order to account for this, species data were converted to proportions of the full species count.

In addition, the following summary data were collated for each bulked sample:

1) The minimum possible number of species present (described as minimum species richness or MSR; i.e. all species plus genus and family level groups where there are no higher order members present) within the following groups);
   - The whole assemblage
   - Trichoptera
   - Ephemeroptera
   - Ephemeroptera not of the family Leptophlebiidae (known to be an acid tolerant family)
   - Plecoptera
   - Odonata
   - Hemiptera
   - Coleoptera

3) Total number of individuals within the following groups (identified to any level) expressed as a proportion of the total number of individuals in the sample;
   - Trichoptera
   - Ephemeroptera
   - Plecoptera
   - Trichoptera + Ephemeroptera + Plecoptera
   - Odonata
   - Hemiptera
   - Coleoptera
   - Tricladida
   - Chironomidae

6.3.3 Decision trees

Decision trees are popular in many fields as a way of encapsulating and structuring the knowledge of experts for use by the less experienced. They are commonly used in botany and medical decision making for example. Automatic tree construction was first developed in the social sciences, but the work of Breiman and colleagues in the late 1970's and early 1980's (encapsulated in their monograph; Breiman et al., 1984) placed tree-based models firmly within a statistical framework. Since then, the properties of tree-based classifiers have been well studied and are widely regarded as being a powerful tool for supervised classification purposes. Recent advances such as bagging (Breiman 1996), boosting (Freund and Schapire, 1997) and random forests (Breiman, 2001) have been developed that extend the tree concept to so-called ensemble methods to improve predictions from trees, but do so at the expense of simplicity and, to some extent, interpretability.

Tree-based methods partition the “feature space” into a set of regions and then fit a simple model, such as a constant one, in each one. Tree-based models are computationally intensive methods that are ideally suited to situations where there are many explanatory variables to choose from and it is not known *a priori* which of them are most important. The main virtues of tree-based models are that they are that they are excellent for initial data inspection, they give a clear picture of the structure of the data and they provide a highly intuitive insight into the kinds of interactions between variables.

Models are fitted using binary recursive partitioning, where the data are successively split along features of the environmental data so that at any node the split which maximally distinguishes the response variable in the left and right branches is selected. Splitting continues until the nodes are "pure", i.e. comprising one class only, or the data are too sparse.

Where the response variable is a factor (i.e. grouped in classes), the tree is known as a classification tree. Where the response is continuous, the tree is known as a regression tree. Because the recursive partitioning continues until pure nodes are reached or the samples in each node are too sparse, there is a danger of over fitting the response. Tree-based models are generally pruned back to a minimal, adequate model. This is done via a cross-validation procedure to obtain "honest" estimates of the true prediction error. Plotting this prediction error against tree-size allows the selection of the tree with the minimum error. An alternative is to select, as the best tree, the smallest tree whose estimated error rate is within one standard deviation of the minimum error rate. A simple introduction to the use of classification and regression trees in ecological data analysis is given by De'ath and Fabricius (2000).

In this report classification trees were used to predict a class status for High-Good, Moderate and Poor/Bad from the macroinvertebrate training set data:

Trees were fitted in the R computer software (Version 2.5.1; R Core Development Team 2007) using the rpart package by Therneau and Atkinson (2007). Splits were determined via minimising the Gini index measure of node impurity:

$$D_i = 1 - \sum_k p_{ik}^2$$

where p_{ik} is the observed proportion of class k within node i, and D_i is the node impurity for node i. The total measure of node impurity is then:

$$\sum D_i$$

Trees were fitted to their maximal extent and then a 10-fold cross-validation (CV) procedure was used to identify the smallest size of tree within 1 standard error of the tree with the lowest cross-validated misclassification error. In 10-fold CV, the training data are randomly assigned to one of ten groups. In turn, each group is excluded from the CV training set whilst the remaining nine groups are used to grow an "unpruned" tree. This tree is then used to predict the class membership of the samples in the group left out. This is repeated for each group in turn. At each of the 10 stages, the misclassification error is computed for each tree of size n, n = 1, ..., m, m = number of samples. The average error across the 10 CV stages is used as a measure of tree performance, with an associated standard error.

Predictions from the tree are governed by the terminal nodes or leaves of the tree. Predictions are based on a "majority rules" concept, whereby the predicted class for a target sample is determined by the most abundant class of the node the target sample ends up in. The proportion of samples classified into a particular node can also be used as an indicator of class probability; a target sample ending up in a node containing samples mainly of class k will have a high probability of belonging to class k. Conversely, a target sample ending up in a node with a mixture of classes will have a lower probability of belonging to the majority class.

Due to the uneven sampling of sites within each class and a desire to minimise "High" or "Good" status sites being classified as less than "Moderate" or worse (and vice versa), two additional parameters were used to grow the tree.

The prior probabilities of class membership are one of the important parameters controlling tree growth. If these are unspecified, the prior probabilities are taken as the number of samples in each class expressed as a proportion of the total number of samples in the training set. Given the unbalanced structure of our dataset, simply by assuming that any site was in High-Good class one would be correct over 50% of the time – a considerably higher probability than randomly guessing site class. In data sets that are heavily biased to one or a few classes, the priors will be biased towards well-represented classes and as such misclassification rates can be minimised by concentrating on correctly assigning the well-represented classes at the expense of the poorly-represented ones. This will often result in low misclassification rates for the well represented classes but high misclassification rates for the poorly represented classes. In effect, the classification tree will be a poor predictor of class for the poorly-represented classes.

To balance weights of each class in the fitting algorithm, we defined the prior probability of belonging to a given class to be 0.33 for all three classes. This means that, *a priori*, any sample has an equal chance of belonging to any of the three classes.

Variable misclassification costs can be used by supplying the fitting routine with a loss matrix. This loss matrix describes the relative "costs" of classifying a site of a known class to a different class. By specifying the relative misclassification costs in a loss matrix, we can control the fitting algorithm by penalising certain types of misclassification more strongly than others. For example, in the case of WFD classification, classifying a "Poor-Bad" site as "Moderate" may be considered less costly than classifying it as "High-Good".

The loss matrix used was:

site class	***prediction***		
	High-Good	**Moderate**	**Poor-Bad**
High-Good	0	3	3
Moderate	3	0	3
Poor-Bad	3	1	0

The diagonal elements of the loss matrix are equal to 0, indicating that there is no misclassification cost associated with correctly classifying a sample. The off-diagonal elements of the loss matrix describe the relative misclassification costs. Here we have said that classifying a "High-Good" site as "Moderate" (or vice versa) incurs a relative misclassification cost of 3 – this is the default misclassification cost in the rpart fitting algorithm. The value 3 was chosen simply to add additional penalty to this type of misclassification – there is no underlying theoretical justification for choosing this value, but the relative misclassification costs used appeared to balance the per class error rates and limit the number of "High-Good" sites being classified as "Moderate" or worse and vice versa.

7 WFD60 RESULTS

7.1 WFD60 Classification

Preliminary runs of the tree using all biological parameters listed in Section 6.3.2 demonstrated that species-level data were superfluous in the discrimination of physico-chemical classes. Variables representing the minimum species richness (MSR) of the entire assemblage and the MSR of non-leptophlebid Mayflies particularly, were found to be extremely powerful predictors. A tool based on such generic-level indicators is likely to be more robust against taxonomic errors than one based predominantly on presence/absence/abundance of individual species. The final run of the tree, therefore was based on MSR groupings, and proportions of individuals from the major taxonomic groups only.

The output from the classification tree, based on the damage matrix illustrated in Table 6.1, is provided in Figure 7.1. The Figure illustrates the nodes (circles) and terminal nodes (boxes) of the tree. Class status (i.e. HIGH-GOOD, MODERATE or POOR-BAD) is given in each node and terminal node and represents the “posterior probability”, that a site of a physico-chemically defined class will fall into that node.

The first split in the tree is defined by a MSR (i.e. the minimum possible number of taxa present in the sample) of thirteen or more. The majority of sites with an MSR >12.5 fall into a node classed HIGH-GOOD, which also contains 15 sites defined physico-chemically as MODERATE. The relationships between MSR and ANC and Ca^{2+} are provided in Figures 7.2 and 7.3 respectively. Figure 7.2 illustrates the very rapid decline in MSR as ANC falls toward zero.

The next split on this branch is dependent on the MSR for Mayfly taxa not of the family Leptophlebiidae. Those sites with one or more of these Mayflies fall into another node classed HIGH-GOOD. 62 of the sites in this node are correctly classified, while another 3 are of the physico-chemical class “Moderate”. The relationships between non-leptophlebid Mayfly taxa numbers and ANC and Ca^{2+} are provided in Figures 7.4 and 7.5 respectively. Like MSR, this criterion shows a very steep response as ANC declines toward zero. However, unlike MSR, there is no strong relationship with Ca^{2+}. This suggests that acid sensitive Mayflies may be abundant in highly sensitive waters, provided these are not acidified.

Continuing with this sub-branch, a further split determines that sites where the combined abundance of Ephemeropteran, Plecopteran and Trichopteran (EPT) individuals comprise 25% or more of the assemblage, fall into a terminal node classed HIGH-GOOD. In summary, all 55 sites in this training set with an MSR of thirteen or more, at least one non-leptophlebid Mayfly and an assemblage comprising at least 25% EPT individuals fall into the class HIGH-GOOD and no site is mis-classified. The plots in Figures 7.6 to 7.7 show no obvious relationships between this metric and the key chemical variables, suggesting this split is, very much, a secondary criteria. It may operate to downgrade sites with relatively high diversity that have very high numbers of chironomids and oligochaetes – a feature of some moderately acidified lakes. Overall, however, the combination of high diversity, the inclusion of one of the most acid-sensitive groups (i.e. sensitive Mayflies), and a metric based on the ratio of EPT taxa to the wider assemblage, as criteria for determining at least “Good” status is entirely consistent with WFD normative definitions..

Those sites with fewer than 25% EPT individuals on this sub-branch fall into a MODERATE node. This is split further on the basis of the number of Plecopterans in these samples. Those sites with fewer than 2 Plecopteran taxa (inferring that the bulk of the EPT taxa are likely to be either or Ephemeropterans and Plecopterans) are returned to a HIGH-GOOD terminal node. Six sites reach this terminal node and all are correctly classified. Those sites with two or more

Plecopteran taxa fall are placed in MODERATE terminal node. Plots of relationships are provided in Figures 7.8 and 7.9.

Sites with an MSR >12.5 but no Mayfly taxa that are not leptophlebids fall into a terminal node classed MODERATE. This is also consistent with WFD normative definitions that require evidence that “major taxonomic groups” (in this case all acid-sensitive Mayfly taxa) are lost as the GOOD-MODERATE boundary is crossed. However, there is some mis-classification here, with twelve sites which are considered to be of “High” to “Good” status physicochemically being included in this terminal node. It is of course feasible that factors other than acidification may have important influences on the macroinvertebrate assemblages of these sites that cause them to deviate from what we might consider to be “High” to “Good” status biologically.

Returning to the first split in the tree, sites with fewer than 13 taxa fall into a node classed POOR-BAD. However, a further split determines that of these sites, those with at least one bivalve mollusc taxa present eventually fall into a MODERATE terminal node. Those sites without bivalve molluscs are placed in the only POOR-BAD terminal node. All nine sites we ranked as “Poor” to “Bad” on the basis of their physico-chemistry, end up in this node which also contains four sites we determined were of “High” to “Good” status physico-chemically. Relationships between this metric and ANC and Ca^{2+} are presented in Figures 7.10 and 7.11. Once again, the POOR-BAD classification is entirely consistent with normative definition requirements for low biodiversity and the absence of major taxonomic groups. However, after differentiation of sites on the grounds of species richness there is little requirement from the tree to use further characteristics of the assemblage to make classification decisions. On inspection, the assemblage of all nine sites that were correctly classified is dominated, almost solely, by acid tolerant Plectopterans and chironomids in all cases. Figure 7.10 shows that bivalves occur frequently across the dataset but are not found in any site with a mean ANC less than zero.

Figure 7.1 Classification tree based on the 105 lakes in the WFD60 training set and acidification damage matrix provided in Table 6.1.

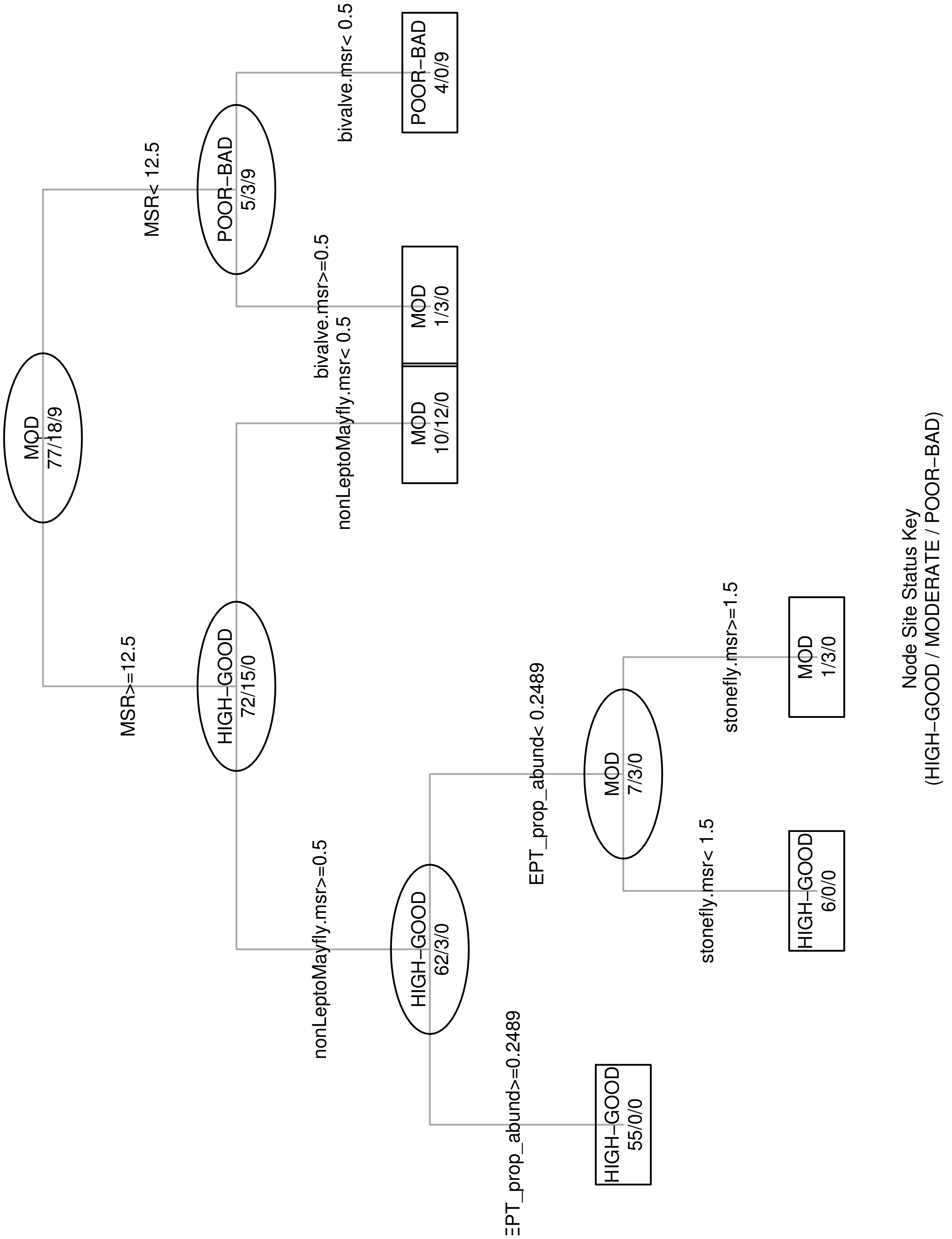


Figure 7.2 Minimum species richness (MSR) on a gradient of ANC for the 105 lakes in the WFD60 training set. Lines represent a GAM function for the count data (Poisson errors, log link function).

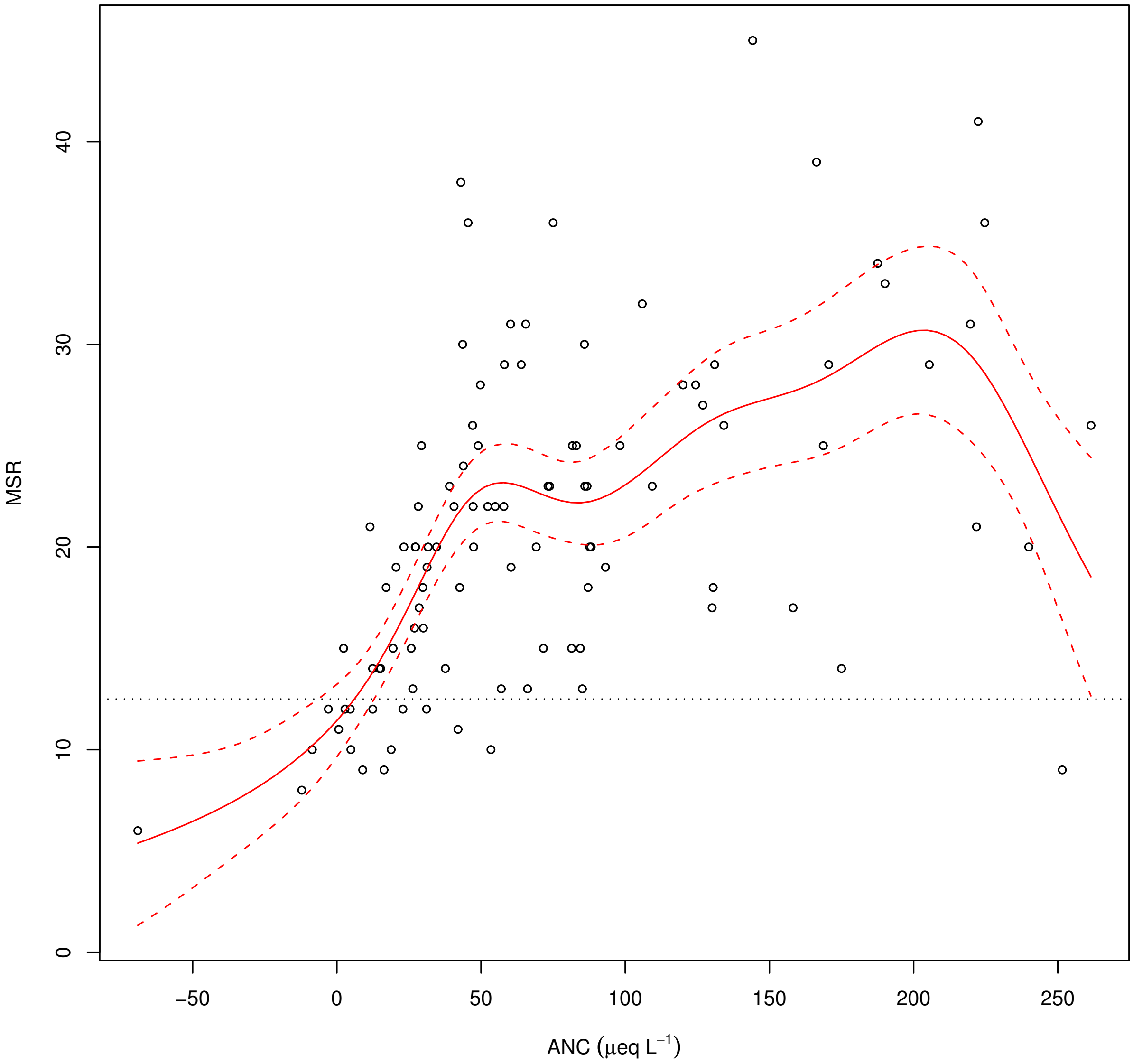


Figure 7.3 Minimum species richness (MSR) on a gradient of calcium concentration for the 105 lakes in the WFD60 training set. Lines represent a GAM function for the count data (Poisson errors, log link function).

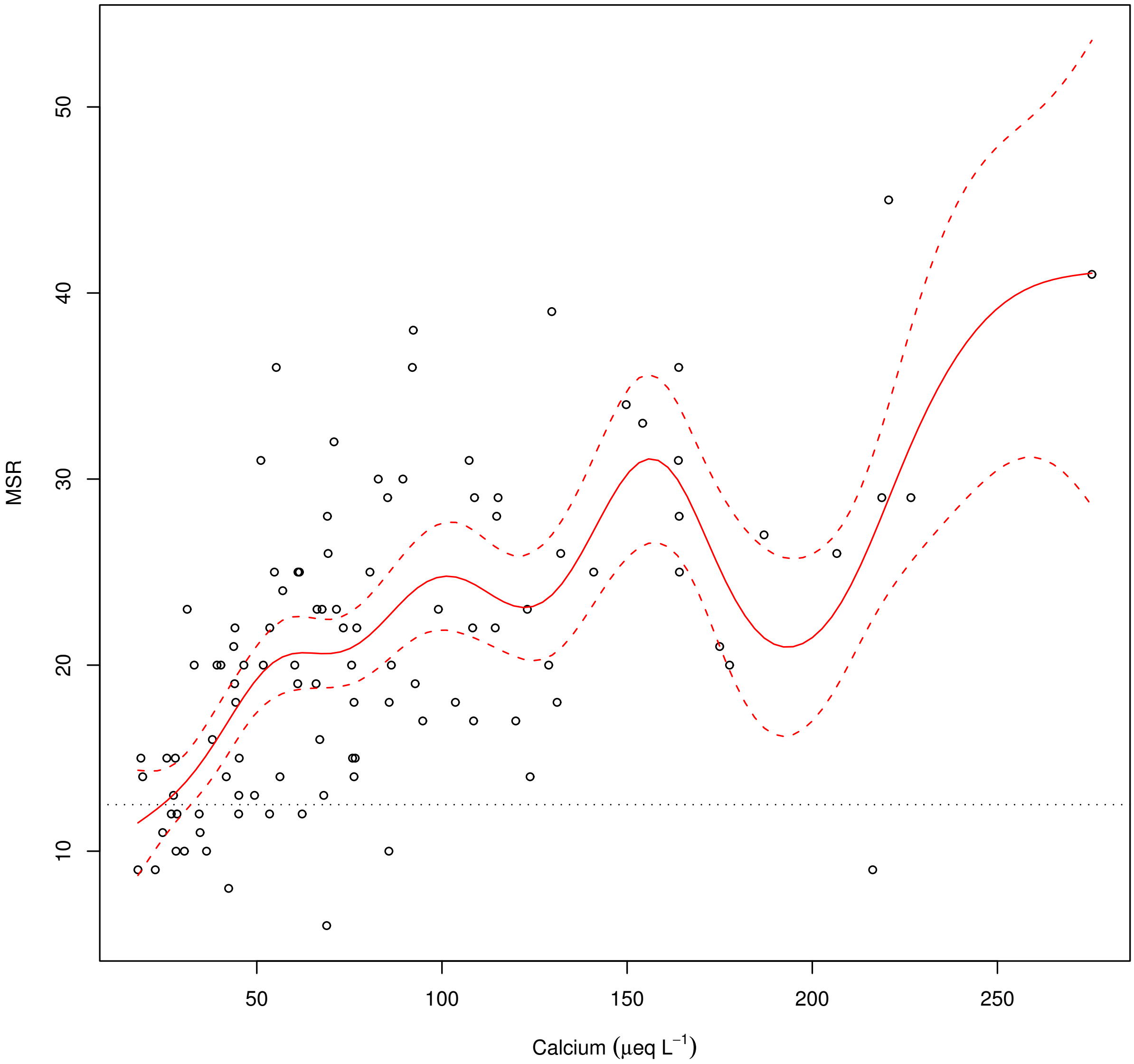


Figure 7.4 Mininium number of Mayfly taxa for each site that are not in the family Leptophlebiidae (nonLeptoMayfly.MSR) on a gradient of ANC for the 105 lakes in the WFD60 training set. Lines represent a GAM function for the count data (Poisson errors, log link function).

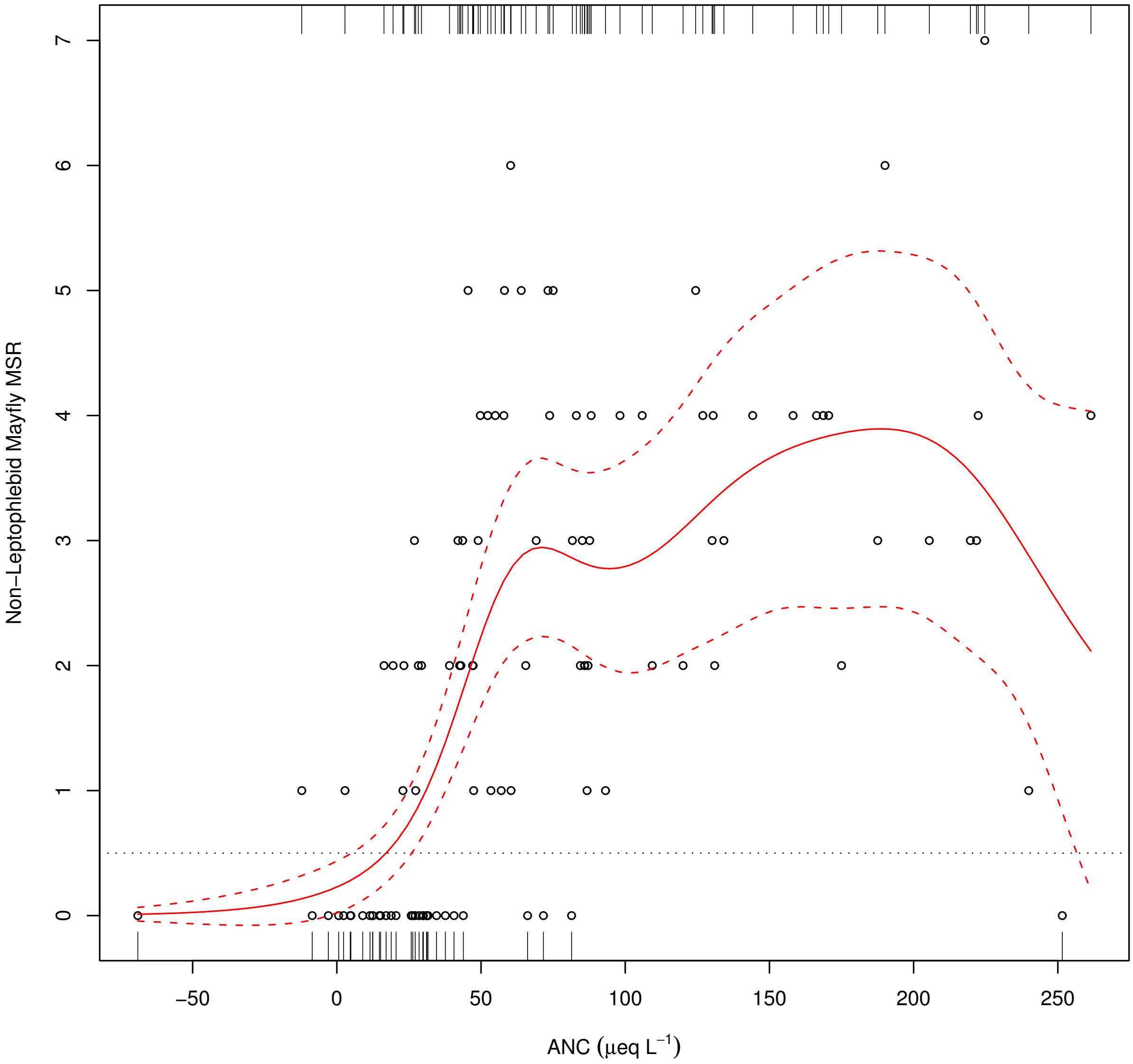


Figure 7.5 **Mininium number of Mayfly taxa for each site that are not in the family Leptophlebiidae (nonLeptoMayfly.MSR) on a gradient of calcium concentration for the 105 lakes in the WFD60 training set. Lines represent a GAM function for the count data (Poisson errors, log link function).**

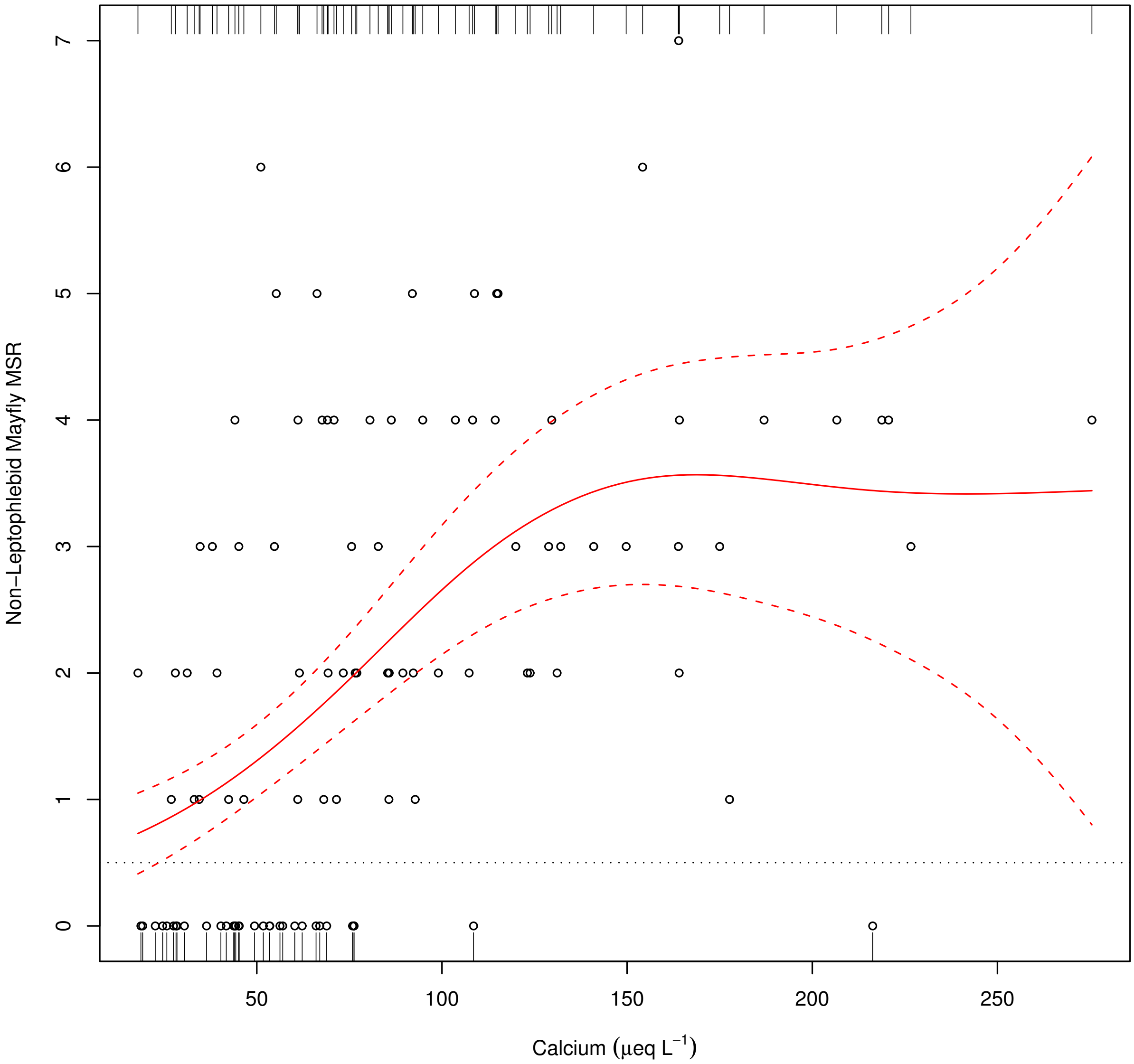


Figure 7.6 The proportion of all individuals for each site that are either Ephemeroptera, Plecoptera or Trichoptera on a gradient of ANC for the 105 lakes in the WFD60 training set. Lines represent a GAM function for the count data (Poisson errors, log link function).

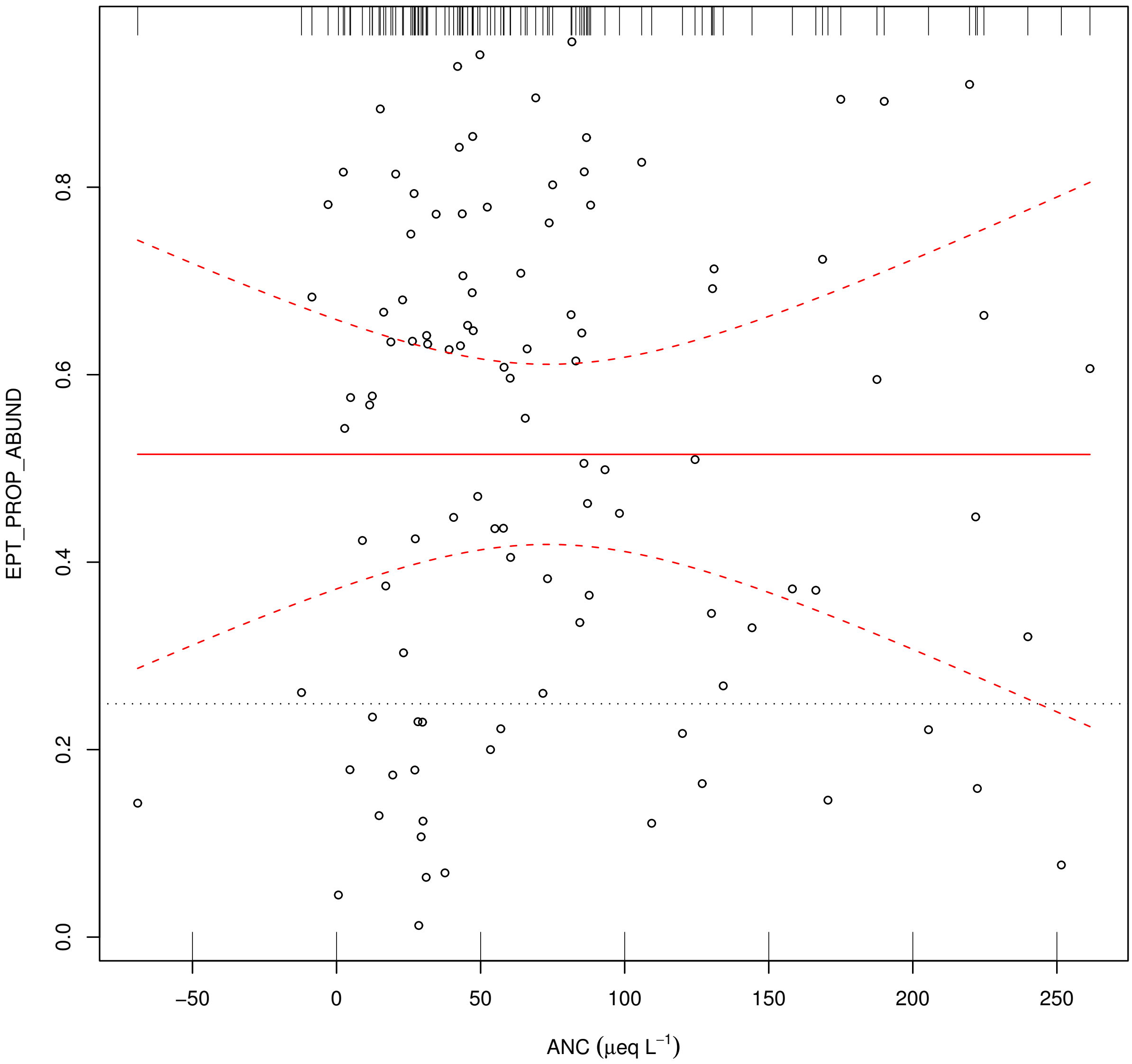


Figure 7.7 **The proportion of all individuals for each site that are either Ephemeroptera, Plecoptera or Trichoptera on a gradient of calcium concentration for the 105 lakes in the WFD60 training set. Lines represent a GAM function for the count data (Poisson errors, log link function).**

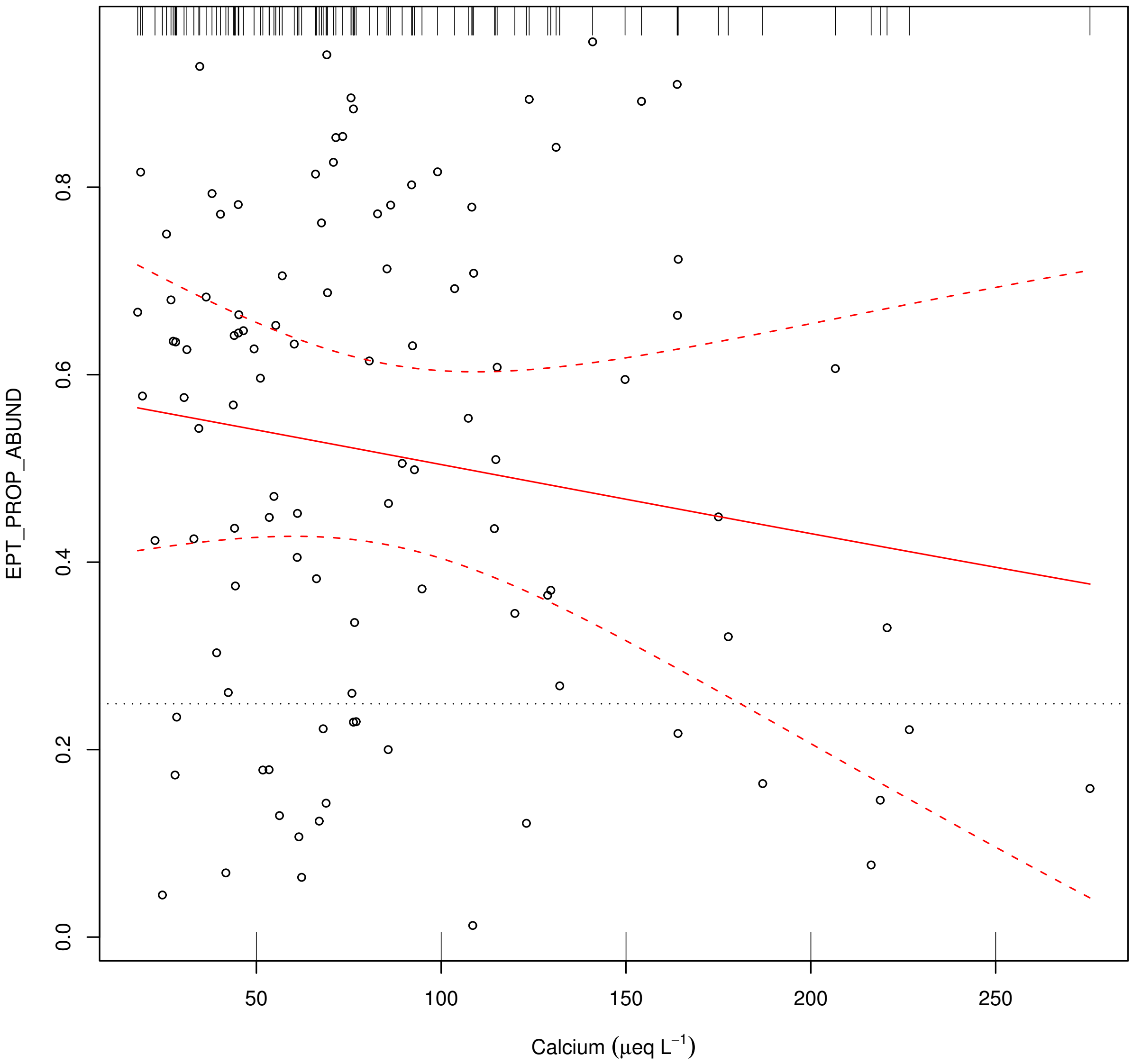


Figure 7.8 The minimum number of stonefly species for each site a gradient of ANC for the 105 lakes in the WFD60 training set. Lines represent a GAM function for the count data (Poisson errors, log link function).

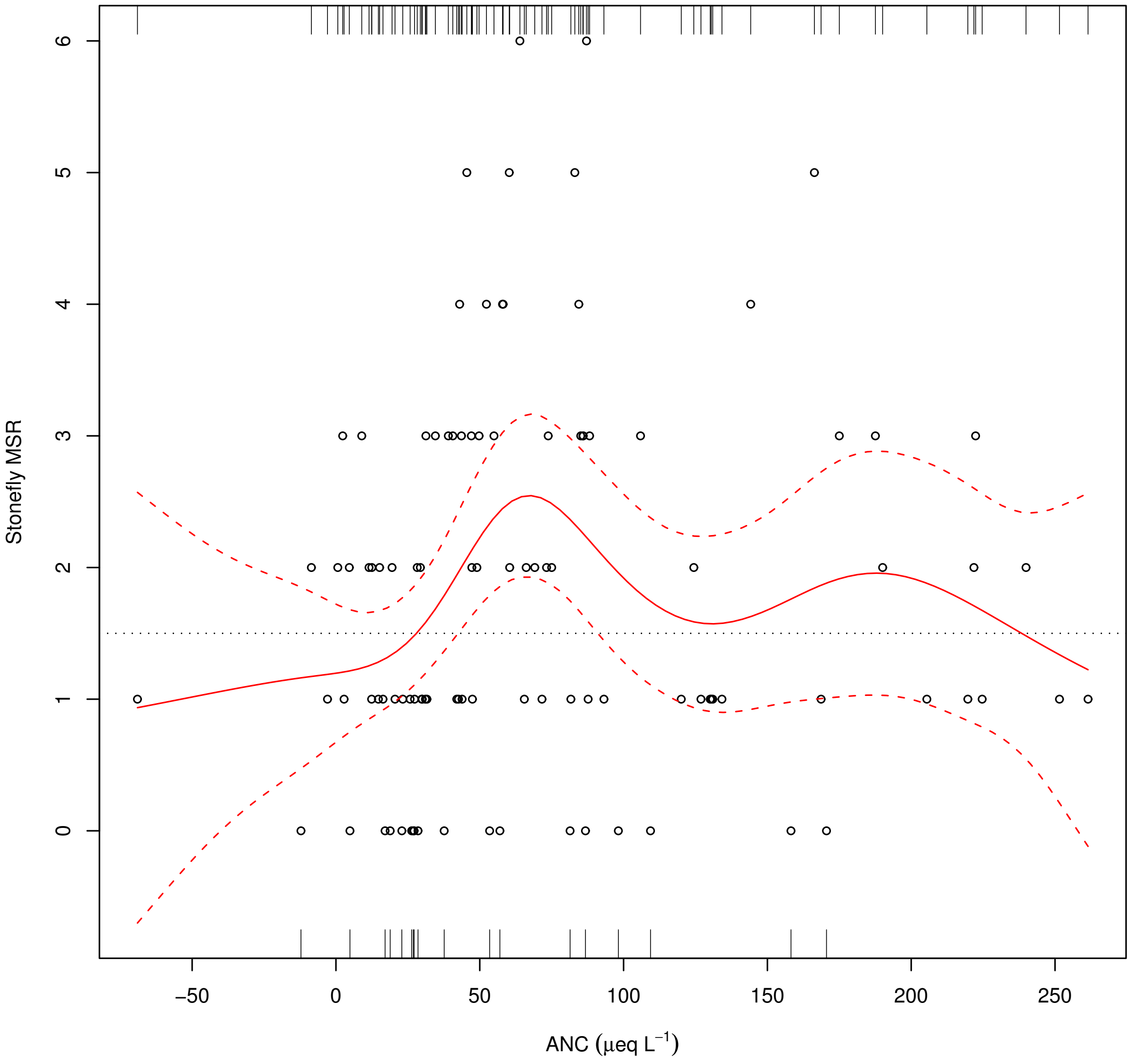


Figure 7.9 The minimum number of stonefly species for each site a gradient of calcium concentration for the 105 lakes in the WFD60 training set. Lines represent a GAM function for the count data (Poisson errors, log link function).

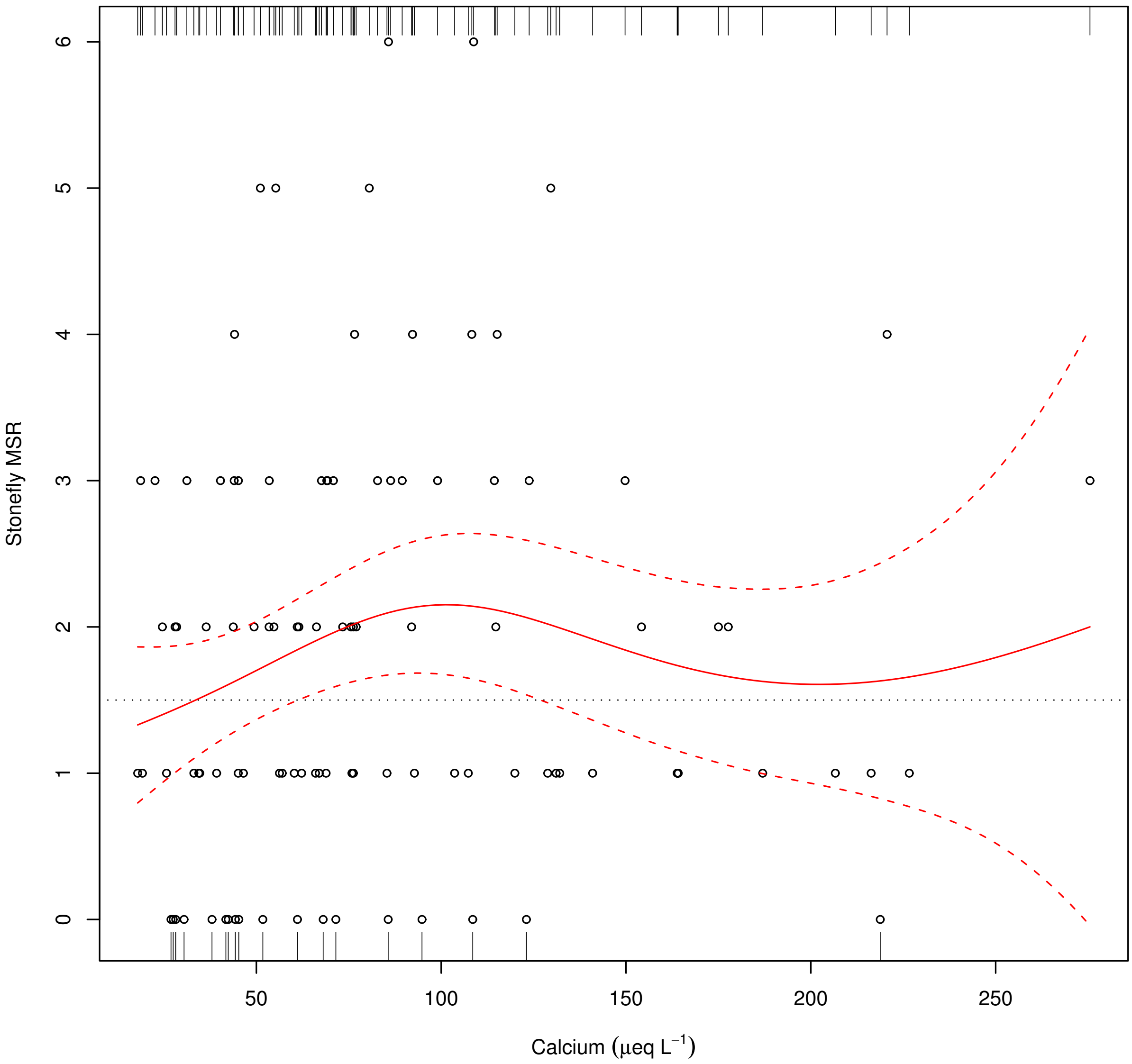


Figure 7.10 The minimum number of bivalve taxa for each site on a gradient of ANC for the 105 lakes in the WFD60 training set. Lines represent a GAM function for the count data (Poisson errors, log link function).

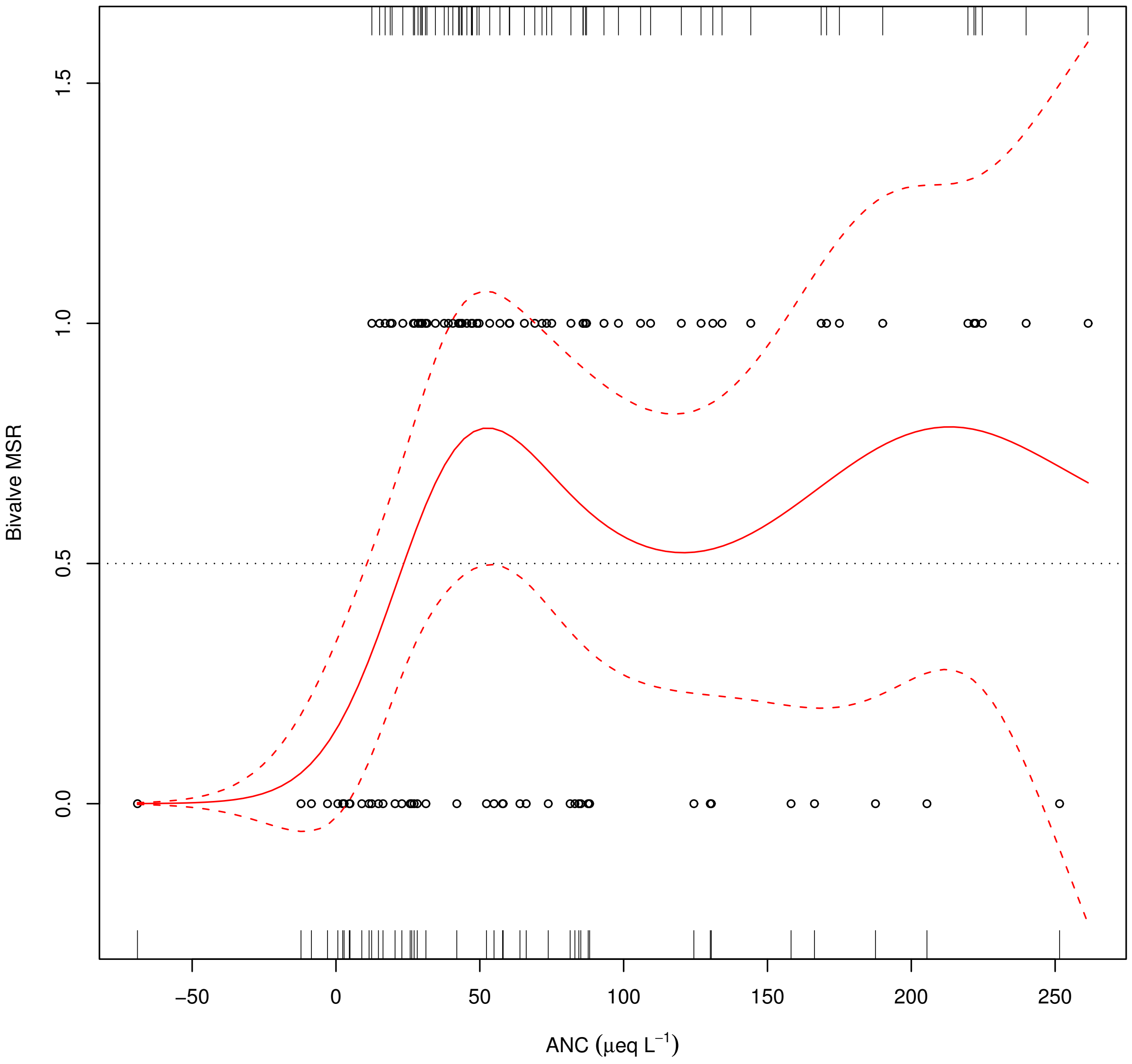
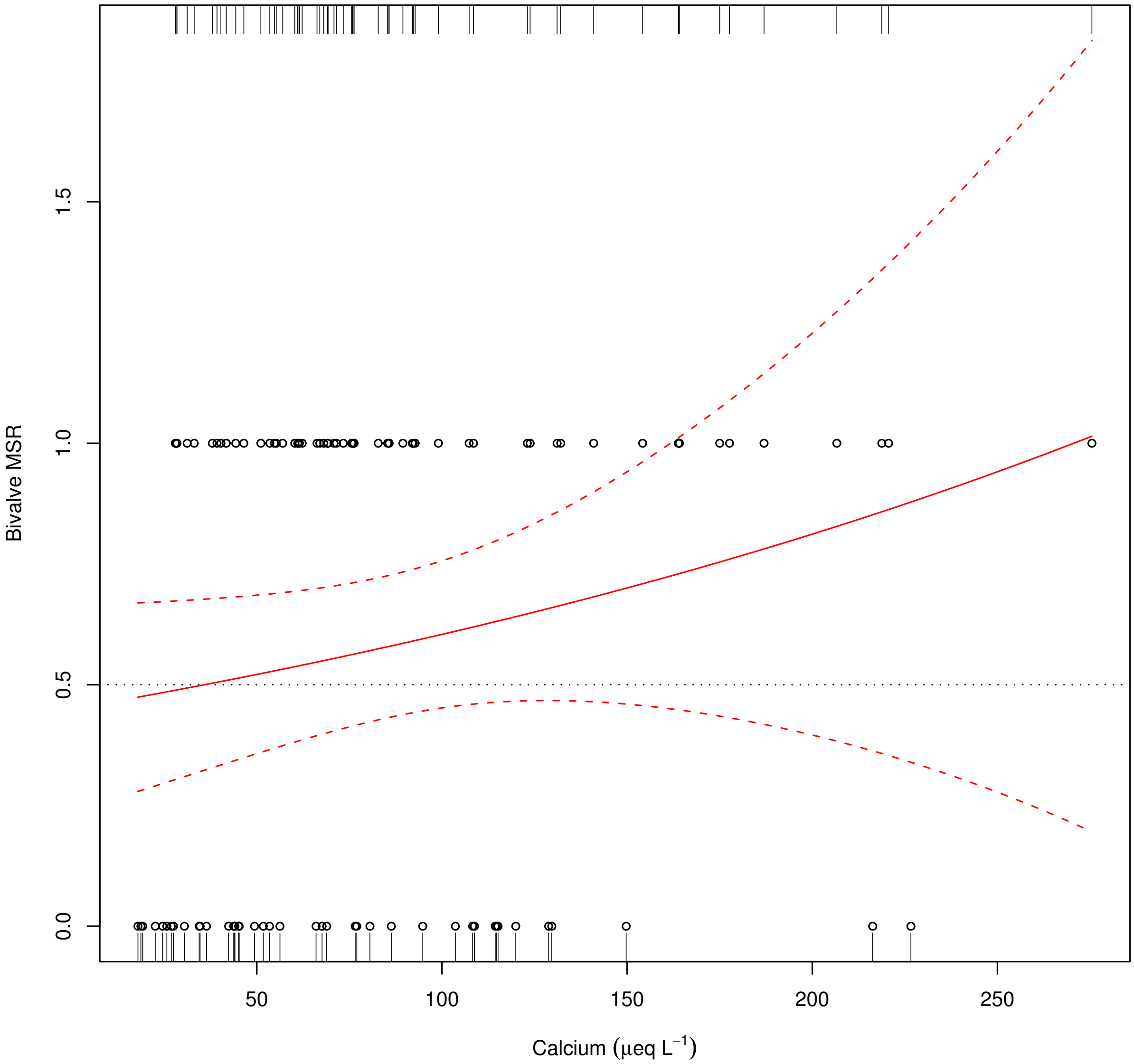


Figure 7.11 The minimum number of bivalve taxa for each site on a gradient of calcium concentration for the 105 lakes in the WFD60 training set. Lines represent a GAM function for the count data (Poisson errors, log link function).



The fitted classification tree correctly classified training set samples 81.7% of the time, meaning it had an error rate of 18.3%. This error rate is a biased statistic, as it is based on data used to fit the model. This will always be an overly optimistic assessment of model error.

The .632+ bootstrap procedure and bagging produced similar estimates of future error rates of 22% and 23% respectively. This indicates that the tree is likely to correctly assign class status to a sample 78% and 77% of the time respectively. The .632+ procedure is indicative of the error of using the final fitted tree for future predictions. The slightly higher success rates of the bagging procedure reflects the observation that ensembles of poor classifiers can often out-perform the single best classifier. In this case, bagging provides a modest improvement in model performance but at the expense of ease of model interpretation.

The cross-validated error rates are not much below that of the apparent error rate of the fitted tree. This suggests that the use of the tree is reasonably robust for predicting the class status of new sites that were not included in the training set. This level of success is encouraging given the small data set and biased sampling of classes.

The cross-classification table for the final tree is shown in Table 7.1 The largest error is for the 12 sites of “High” to “Good” physico-chemical status that are incorrectly classed as MODERATE by the tree. Four further sites from the top physico-chemical group are classed as POOR-BAD. Otherwise the simple tool is able to correctly attribute all sites physico-chemically defined as “Moderate” or “Bad” to the appropriate class on the basis of a small number of biological characteristics.

The success rate for the sites of “High” to “Good” physico-chemistry is disappointing, but from a precautionary Water Framework Directive perspective it could be argued that it is better to incorrectly identify a clean site as being in an acidified condition (a kind of Type II error), than pass a moderately, poorly or badly acidified site as being in an unacidified condition (i.e. a Type I error).

Table 7.1 WFD60 Tree Cross-classification Table

Predicted class	**Physico-chemical Class**		
	High	Moderate	Poor-Bad
HIGH-GOOD	61	0	0
MODERATE	12	18	0
POOR-BAD	4	0	9
Success Rate (%)	79.2	100.0	100.0

Table 7.2 shows the posterior class probabilities for the terminal nodes (leaves) of the final fitted classification tree. The terminal nodes are numbered from left to right on the tree. For node 6 for example, the assigned class is POOR-BAD with a posterior probability of 0.95. There is a 0.00 posterior probability of the predicted class being MODERATE, and 0.05 posterior probability of being HIGH-GOOD.

Table 7.2 Tree Posterior Class Probabilities

Node (class)	High-Good	Moderate	Poor-Bad
1 (HIGH-GOOD)	1.00	0.00	0.00
2 (HIGH-GOOD)	1.00	0.00	0.00
3 (MODERATE)	0.07	0.93	0.00
4 (MODERATE)	0.16	0.84	0.00
5 (MODERATE)	0.07	0.93	0.00
6 (POOR-BAD)	0.05	0.00	0.95

8. A GEOGRAPHIC TEST OF THE CURRENT TOOL

The WFD60 database contains many more samples, and sites, than have been included in the training set. This is because there has often been insufficient water chemistry data of sufficient quality to provide annual mean chemistry estimates for these biological samples.

While these additional macroinvertebrate samples cannot, therefore, be used to test the tool quantitatively, it is still useful to inspect the geographical distribution of classification of these independent samples. Given our understanding of the geographic distribution of acidified lakes in the UK we would expect that, in general, lakes in northwest Scotland would normally be in Good to High status, whereas sensitive lakes in parts of Snowdonia, the Pennines, Galloway, the Trossachs and the Cairngorms would be more likely to class as Poor-Bad or Moderate.

Class predictions for new sample/sites are made by introducing each new sample at the top of the tree and propagating it through the tree structure following the appropriate branches until a terminal node is reached. The predicted class for the new sample is the class of the terminal node in which it ends up. The posterior probability of the sample belonging to the predicted class, given that it is predicted to be that class, is simply the posterior probability of the classes for the node of the tree based on the proportions of training samples on different classes in located in that node.

Of the 72 sites in this test, 39 were classed High-Good, 20 were classed Moderate and 13 were classed Poor-Bad by the WFD60 tool The geographic distribution, shown in Figure 8.1 complies well with expectations although there are some surprising results. For example, Loch Grannoch samples contained 15 taxa and a non-leptophlebid Mayfly and this site was therefore classed as HIGH-GOOD, despite its high Al_{lab} levels and low levels of ANC. At the opposite extreme, Llyn Cau, which shows no palaeoecological evidence of having acidified is classed as POOR-BAD.

The sites in this test are listed in Table 8.1 together with their posterior probabilities of class. These results are promising, but clearly the tool requires further tuning before it is capable of repeatedly delivering appropriate classes.

Figure 8.1 UK Map of the WFD60 classification tree classification of 51 lakes. Detail provided in Table 8.1.

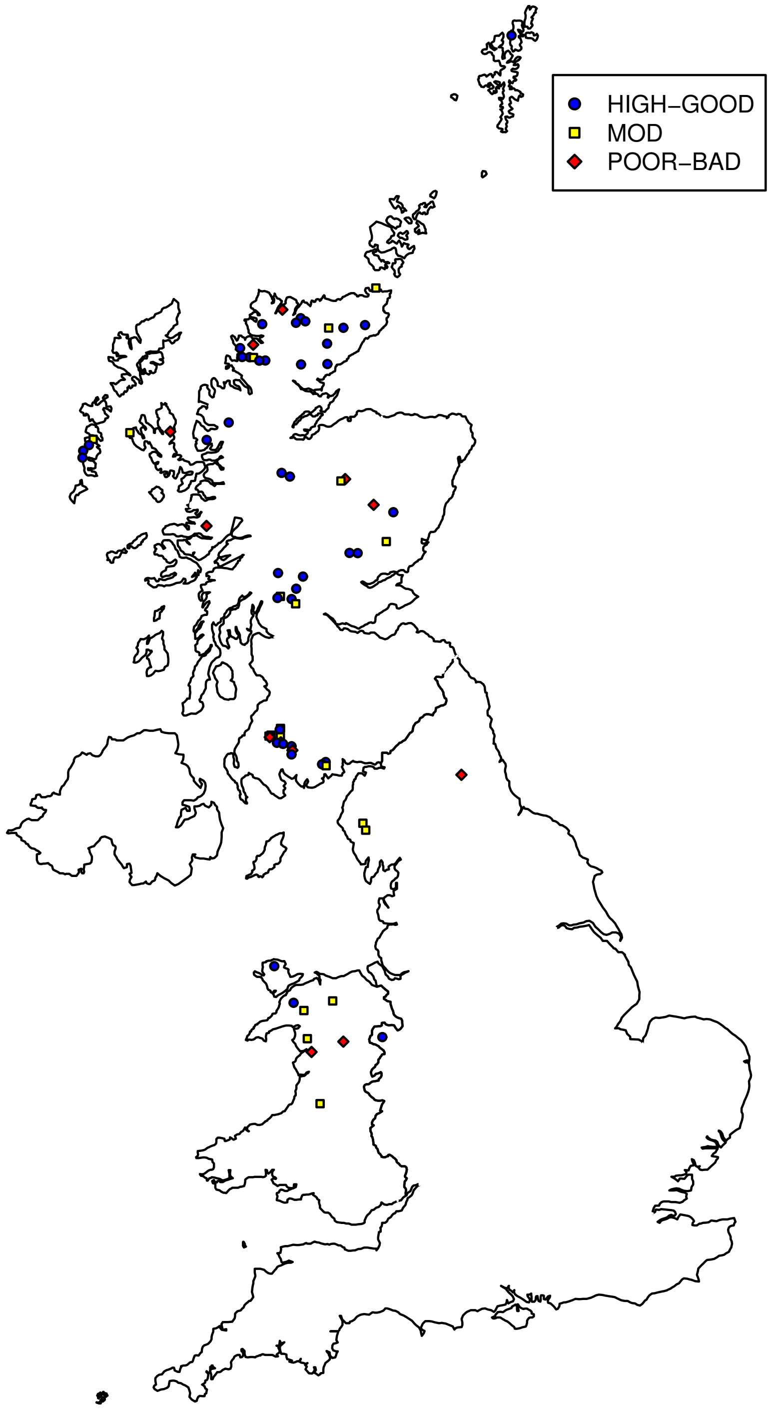


Table 8.1 Sites used to test the WFD60 tool, resulting classification and posterior probabilities derived from the classification tree.

WBID	SITE	GRID REF	Class	HIGHGOOD	MOD	POORBAD
27967	Clatteringshaws	NX542770	HIGH-GOOD	1	0	0
34002	Cwellyn	SH559549	HIGH-GOOD	1	0	0
28345	Dalbeattie Reservoir	NX806613	HIGH-GOOD	1	0	0
28302	Edingham Loch	NX837633	HIGH-GOOD	1	0	0
32538	Llyn Alaw	SH392866	HIGH-GOOD	1	0	0
34002	Llyn Cwellyn	SH559549	HIGH-GOOD	1	0	0
11338	Loch Ailsh	NC315109	HIGH-GOOD	1	0	0
19593	Loch Aird An Sgairbh	NF734268	HIGH-GOOD	1	0	0
8945	Loch Ascaig	NC849255	HIGH-GOOD	1	0	0
11355	Loch Borralan	NC262108	HIGH-GOOD	1	0	0
11611	Loch Brora	NC852078	HIGH-GOOD	1	0	0
235	Loch Calder	HU444922	HIGH-GOOD	1	0	0
24754	Loch Chon	NN420053	HIGH-GOOD	1	0	0
16443	Loch Clair	NG999572	HIGH-GOOD	1	0	0
23561	Loch Clunie	NO114441	HIGH-GOOD	1	0	0
18209	Loch Coire Nan Arr	NG808421	HIGH-GOOD	1	0	0
5307	Loch Coulside	NC580435	HIGH-GOOD	1	0	0
11642	Loch Craggie	NC624074	HIGH-GOOD	1	0	0
23557	Loch Craiglush	NO043443	HIGH-GOOD	1	0	0
9669	Loch Culag	NC097216	HIGH-GOOD	1	0	0
27948	Loch Dee	NX469790	HIGH-GOOD	1	0	0
18682	Loch Druidibeag	NF789376	HIGH-GOOD	1	0	0
24798	Loch Drunkie	NN542043	HIGH-GOOD	1	0	0
24132	Loch Earn	NN641238	HIGH-GOOD	1	0	0
28130	Loch Grannoch	NX542699	HIGH-GOOD	1	0	0
24056	Loch Iubhair	NN425269	HIGH-GOOD	1	0	0
20601	Loch Killin	NH528104	HIGH-GOOD	1	0	0
20465	Loch Knockie	NH456135	HIGH-GOOD	1	0	0
21945	Loch Lee	NO422795	HIGH-GOOD	1	0	0
3904	Loch Loyal	NC621475	HIGH-GOOD	1	0	0
24459	Loch Lubnaig	NN582133	HIGH-GOOD	1	0	0
27699	Loch Macaterick	NX440914	HIGH-GOOD	1	0	0
5714	Loch Rangag	ND177415	HIGH-GOOD	1	0	0
10786	Loch Sionascaig	NC114139	HIGH-GOOD	1	0	0
5350	Loch Stack	NC288424	HIGH-GOOD	1	0	0
4974	Loch Syre	NC661448	HIGH-GOOD	1	0	0
27936	Loch Trool	NX415799	HIGH-GOOD	1	0	0
10719	Loch Veyatie	NC178137	HIGH-GOOD	1	0	0
6236	Lochan Airigh Leathaidh	NC990392	HIGH-GOOD	1	0	0
19170	West Loch Ollay	NF740327	HIGH-GOOD	1	0	0
29215	Burnmoor Tarn	NY183043	MOD	0.163	0.837	0
10934	Cam Loch	NC213134	MOD	0.163	0.837	0
24919	Lake Of Menteith	NN577003	MOD	0.072	0.928	0
33962	Llyn Alwen	SH897565	MOD	0.163	0.837	0
35578	Llyn Cwm Mynach	SH679238	MOD	0.163	0.837	0
38394	Llyn Hir	SN789676	MOD	0.163	0.837	0
34319	Llyn Llagi	SH649482	MOD	0.163	0.837	0
6234	Loch Culaidh	NC863390	MOD	0.163	0.837	0
27808	Loch Enoch	NX445851	MOD	0.163	0.837	0
27693	Loch Fannie	NX446924	MOD	0.163	0.837	0

23024	Loch Kinnord	NO361541	MOD	0.072	0.928	0
27778	Loch Kirriereoch	NX364866	MOD	0.163	0.837	0
27785	Loch Moan	NX347857	MOD	0.163	0.837	0
17514	Loch Mor	NG144484	MOD	0.072	0.928	0
2088	Loch Of Mey	ND271736	MOD	0.072	0.928	0
28370	Loch Plantain	NX841601	MOD	0.163	0.837	0
18113	Loch Shnathaid	NF826426	MOD	0.163	0.837	0
24745	Loch Tinker	NN445067	MOD	0.163	0.837	0
20826	Lochan Dubh A Chadha	NH969066	MOD	0.163	0.837	0
29153	Scoat Tarn	NY159103	MOD	0.163	0.837	0
28519	Derwent Reservoir (Northumbria)	NZ011522	POOR-BAD	0.049	0	0.951
35568	Lake Vyrnwy	SH990213	POOR-BAD	0.049	0	0.951
36267	Llyn Cau	SH715123	POOR-BAD	0.049	0	0.951
8751	Loch Assynt	NC210245	POOR-BAD	0.049	0	0.951
22308	Loch Doilet	NM807677	POOR-BAD	0.049	0	0.951
27823	Loch Dow (By Moan)	NX353848	POOR-BAD	0.049	0	0.951
17329	Loch Fada	NG493493	POOR-BAD	0.049	0	0.951
28076	Loch Gower	NX549735	POOR-BAD	0.049	0	0.951
2490	Loch Hope	NC463548	POOR-BAD	0.049	0	0.951
20739	Lochan Na Beinne	NJ006082	POOR-BAD	0.049	0	0.951
21723	Lochnagar	NO252859	POOR-BAD	0.049	0	0.951
27927	Round Loch Of Glenhead	NX449803	POOR-BAD	0.049	0	0.951

9. RECOMMENDATIONS

We have shown that a relatively simple classification approach, based on coarse macroinvertebrate species groupings only, can be used to derive a relatively robust indication of the likelihood of damage to lakes from acidification.

While we accept that the methodology used to derive our proposed classification differs markedly from other proposed UK schemes we believe it offers the most robust way of delivering an ecological meaningful and reliable acidification classification system. As the tool is built on the basis of ecological criteria including diversity and the presence-absence of major taxonomic groups it is suitably compliant with WFD normative definitions outlined in Chapter 1. The tool is also able to discriminate well between acidified and naturally acid (high DOC, low sulphur deposition) lakes, simply because MSR tends to be relatively high in the latter sites which also often support non-leptophlebiid Mayflies.

However we are concerned that the training set used to derive the classification tree is still under-represented by sites at the more acidic and acidified ends of the gradient and we would firmly recommend that the dataset is expanded by between 30-40 sites to increase our confidence of predicting “Moderate” to “Poor” levels of damage.

The most important predictor variable has been found to be minimum species richness (or MSR). Clearly, however, this metric is sensitive to problems of rarerefaction and we intend to investigate alternative expressions of “species richness” to see if this can improve the model. In the meantime, however, it is clear that the degree of sampling effort is important. Data for most sites are amalgamated either from five one-minute kick samples or two three-minute kick samples taken essentially from a single habitat patch. We recommend that the SEPA methodology (i.e. two three-minute kick samples) becomes the standard for any further WFD-related lake macroinvertebrate acidification model development or WFD monitoring.

The tree presented in Figure 8.1 forms the basis of a provisional tool. However, it is highly likely that the specifics of the tool will change, if or when new sites are incorporated. Ultimately it is intended that a physical tool will be produced in electronic format following approval from the UK WFD Lakes Task Team (UK TAG). Currently it is intended that this tool will be in a similar format to that recently developed for the WFD programmes DARES and DALES. There will, therefore, be a requirement for the operator to enter biological data into a spreadsheet, and the tool will then check entries against a species dictionary before delivering the most probable class on the basis of the classification tree splits.

The likelihood that a lake has been assigned the correct class could be assessed further if bagging was included in the tool. However, at present the lack of balance in sites (i.e. the relative paucity of sites that are deemed “Moderate” and “Poor-Bad”) tends to result in different error rates for the three classes and these are difficult to control for within the bagging procedure. Application of bagging will therefore require further raw data to address the current imbalance in the training set.

To date it is not intended for the tool to deliver Ecological Quality Ratios (EQRs) on a continuous scale since our approach is categoric. However, a continuous EQR-compatible scoring system can be developed if bagging is incorporated in the physical tool, based on the relative likelihood that a site would fall into any one class.

Before implementation, we recommend the tool is tested on 1) time series data, to allow an assessment of temporal variability of output, and 2) sites for which detailed multi-proxy biological records are available, so that the macro-invertebrate inferred damage class can be related to wider-ecosystem indications of damage by acidification.

10 CONCLUSIONS

In this report we have presented a novel scheme for a macro-invertebrate-based WFD classification of lake acidification. Classes are derived using a classification tree calibrated using a damage matrix which relates lake physico-chemical indicators of damage to the macroinvertebrate assemblage in accordance with WFD60 normative definitions.

Within the classification tree 82% of samples are allocated the correct class, and we estimate that independent samples will be assigned the correct class in 77-78% of cases. The tree appears to deliver appropriate results for the bulk of 51 independent samples in our test dataset although a minority of sites are obviously incorrectly classified.

This project has been fraught with issues regarding the availability of suitable macroinvertebrate and, more particularly, high quality water chemistry data and its success has been dependent on the availability of data for more acidic sites from ECRC-ENSIS data holdings. The absence of data on labile inorganic aluminium concentration, perhaps the most important ecological factor to limit many macroinvertebrate species has hindered our ability to determine appropriate physico-chemical thresholds for the biota, and it is of concern to us that the UK environmental agencies are not monitoring this determinand routinely in regions threatened and damaged by acidification. The quality of the classification is limited by the paucity of samples representing the acidic and acidified end of the physico-chemical gradient and we recommend that a further 30-40 sites are sampled from this group before any attempt is made to refine the tree. Further data from this group are also necessary if we are to include a component within the WFD60 tool that will provide a probability of correct classification for individual samples.

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APPENDICES

Appendix I Sites and macroinvertebrate sample dates included in the WFD60 training set.

SITE	WBID	Biological data source	DATE
Burnmoor Tarn	29215	AWMN	27/04/2002
Burrator Reservoir	46279	EA	21/03/2006
Buttermere	29052	EA	21/03/2006
Crummock Water	29000	EA	21/03/2006
Derwent Water (Cumbria)	28965	EA	08/03/2006
Dozmary Pool	46232	EA	21/03/2006
Ennerdale Water	29062	EA	21/03/2006
Gloyw Llyn	35233	CLAM	21/04/2002
Grasmere	29184	EA	09/03/2006
Llyn Berwyn	38907	EA	22/03/2006
Llyn Bodlyn	35561	EA	19/04/2006
Llyn Cau	36267	CLAM	20/04/2002
Llyn Clyd	33843	CLAM	23/04/2002
Llyn Conwy	34400	EA	22/03/2006
Llyn Cwellyn	34002	CLAM	21/04/2002
Llyn Cwm Mynach	35578	AWMN	10/04/2002
Llyn Edno	34243	CLAM	22/04/2002
Llyn Egnant	38409	EA	22/03/2006
Llyn Eiddwen	38422	EA	22/03/2006
Llyn Glas	33998	CLAM	22/04/2002
Llyn Gynon	38525	EA	06/04/2006
Llyn Hir	38394	CLAM	21/04/2002
Llyn Idwal	33836	EA	18/04/2006
Llyn Irddyn	35650	CLAM	21/04/2002
Llyn Llagi	34319	AWMN	11/04/2002
Llyn Ogwen	33803	EA	18/04/2006
Llyn Padarn	33730	EA	20/03/2006
Llyn Tegid	34987	EA	22/03/2006
Llyn Teifi	38390	EA	22/03/2006
Loch A' Cham Alltain	5073	CLAM	06/05/2002
Loch A'bhraoin	14293	ENSIS_WFD60	20/04/2005
Loch Achall	12606	ENSIS_WFD60	12/05/2005
Loch Achray	24744	ENSIS_WFD60	02/05/2005
Loch Ailsh	11338	ENSIS_WFD60	05/05/2004
Loch Alvie	20647	ENSIS_WFD60	04/05/2005
Loch An Lagain	12578	ENSIS_WFD60	13/05/2004
Loch Ard	24892	ENSIS_WFD60	02/05/2005
Loch Arkaig	21490	ENSIS_WFD60	26/04/2005
Loch Arron	27849	ENSIS_WFD60	09/05/2006
Loch Bad An Losguiun	20922	CLAM	28/04/2002
Loch Brora	11611	ENSIS_WFD60	17/05/2004
Loch Caslub	18305	ENSIS_WFD60	08/06/2004
Loch Chon	24754	AWMN	03/05/2002
Loch Clair	16443	CLAM	30/04/2002
Loch Coire Fionnaraich	17334	AWMN	04/05/2002
Loch Coire Nan Arr	18209	AWMN	04/05/2002
Loch Doilet	22308	CLAM	27/04/2002

Loch Dubh Camas An	12469	CLAM	01/05/2002
Loch Dughaill	17619	ENSIS_WFD60	16/05/2005
Loch Eilt	21848	ENSIS_WFD60	26/04/2005
Loch Enoch	27808	ENSIS_WFD60	09/05/2006
Loch Gowan	16530	ENSIS_WFD60	17/05/2004
Loch Grannoch	28130	AWMN	03/04/2002
Loch Langabhat	17379		
Loch Lunn Da Bhra	22395	ENSIS_WFD60	29/04/2005
Loch Maree	14057	ENSIS_WFD60	17/05/2004
Loch Meadie (Caithness)	4204	ENSIS_WFD60	18/05/2004
Loch Meiklie	19381	ENSIS_WFD60	24/04/2005
Loch More	6140	ENSIS_WFD60	27/04/2005
Loch Morie	14202	ENSIS_WFD60	12/05/2005
Loch Morlich	20657	ENSIS_WFD60	04/05/2005
Loch Muick	21790	ENSIS_WFD60	12/05/2005
Loch Na Beiste	11238	ENSIS_WFD60	07/04/2004
Loch Na Creige Duibhe	11315	CLAM	02/05/2002
Loch Na Eun	7824	CLAM	04/05/2002
Loch Na Moracha	15316	ENSIS_WFD60	09/06/2004
Loch Nan Gabhar	22577	ENSIS_WFD60	28/04/2005
Loch Narroch	27912	ENSIS_WFD60	10/05/2006
Loch Naver	6405	ENSIS_WFD60	12/05/2004
Loch Neldricken	27872	ENSIS_WFD60	09/05/2006
Loch Ness	18767	ENSIS_WFD60	28/04/2004
Loch Osgaig	11189	ENSIS_WFD60	06/04/2004
Loch Ossian	22259	ENSIS_WFD60	25/04/2005
Loch Rannoch	22782	ENSIS_WFD60	14/04/2005
Loch Ruthven	19540	ENSIS_WFD60	17/05/2004
Loch Stack	5350	ENSIS_WFD60	04/05/2004
Loch Tarff	20633	ENSIS_WFD60	27/04/2005
Loch Tinker	24745	AWMN	03/05/2002
Loch Toll An Lochain	13791	CLAM	01/05/2002
Loch Tormasad	15551	ENSIS_WFD60	09/06/2004
Loch Valley	27900	ENSIS_WFD60	10/05/2006
Loch Venachar	24758	ENSIS_WFD60	02/05/2005
Lochan Bealach Cornaidh	8266	CLAM	03/05/2002
Lochan Coire An Lochain	22125	ENSIS_WFD60	15/05/2006
Lochan Dubh	22223	CLAM	27/04/2002
Lochan Dubh Cadhafuaraich	10307	CLAM	06/05/2002
Lochan Eion	17147	CLAM	30/04/2002
Lochan Feoir	9070	CLAM	04/05/2002
Lochan Fhionnlaidh	11424	CLAM	04/05/2002
Lochan Lairig Cheile	24020	CLAM	25/04/2002
Lochan Na Beinne	20739	ENSIS_WFD60	15/05/2006
Lochan Na H-Achlaise	23361	CLAM	28/04/2002
Lochan Nan Craobh Loisgte	20725	ENSIS_WFD60	15/05/2006
Lochan Nan Geadas	20712	ENSIS_WFD60	15/05/2006
Lochan Nan Nathrach	20735	ENSIS_WFD60	15/05/2006
Lochanan Dubha	11862	CLAM	02/05/2002
Lochindorb	18825	ENSIS_WFD60	28/04/2005
Lochnagar	21723	AWMN	06/05/2002
Long Loch Of Glenhead	27922	ENSIS_WFD60	10/05/2006
Loweswater	28986	EA	08/03/2006

Round Loch Of Glenhead	27927	AWMN	03/04/2002
Scoat Tarn	29153	AWMN	27/04/2002
Tal-Y-Llyn	36405	EA	22/03/2006
Thirlmere	29021	EA	21/03/2006
Wast Water	29183	EA	07/03/2006
White Holme Reservoir	31104	EA	10/04/2006

Appendix II FURSE species codes and shortnames included in the WFD60 training set

Species name	**Furse Code**	**Furse Shortname**
PLANARIIDAE	05110000	PLANARII
POLYCELIS SP	05110200	POLY'LIS
DUGESIA SP	05120100	DUGE'SIA
DUGESIA TIGRINA	05120103	DUGE.TIG
DENDROCOELUM LACTEUM	05130201	DEND.LAC
NEMATODA	10000000	NEMATODA
VALVATA (CINCINNA) PISCINALIS	16130131	VALV.PIS
POTAMOPYRGUS ANTIPODARUM	16140301	POTA.ANT
PHYSA FONTINALIS	16210202	PHYS.FON
RADIX BALTHICA	16220602	RADI.PER
PLANORBIDAE	16230000	PLANORBI
BATHYOMPHALUS CONTORTUS	16230301	BATH.CON
GYRAULUS (GYRAULUS) ALBUS	16230412	GYRA.ALB
GYRAULUS (TORQUIS) LAEVIS	16230421	GYRA.LAE
ANCYLUS SP	16241100	ANCY'LUS
ANCYLUS FLUVIATILIS	16241101	ANCY.FLU
SPHAERIIDAE	17130000	SPHAEMOL
PISIDIUM SP	17130200	PISI'IUM
DREISSENIDAE	17140000	DREISSEN
OLIGOCHAETA	20000000	OLIGOCHA
LUMBRICULIDAE	20110000	LUMCLDAE
LUMBRICULUS VARIEGATUS	20110101	LUMB.VAR
STYLODRILUS HERINGIANUS	20110302	STYL.HER
ENCHYTRAEIDAE	20310000	ENCHYTRA
NAIDIDAE	20330000	NAIDIDAE
STYLARIA LACUSTRIS	20331201	STYL.LAC
TUBIFICIDAE	20340000	TUBIFDAE
LUMBRICIDAE	20420000	LUMCIDAE
EISENIELLA TETRAEDRA	20420401	EISE.TET
GLOSSIPHONIIDAE	22120000	GLOSSIPH
THEROMYZON TESSULATUM	22120201	THER.TES
GLOSSIPHONIA SP	22120400	GLOS'NIA
GLOSSIPHONIA COMPLANATA	22120401	GLOS.COM
HELOBDELLA STAGNALIS	22120701	HELO.STA
ALBOGLOSSIPHONIA HETEROCLITA	22120801	ALBO.HET
ERPOBDELLIDAE	22310000	ERPOBDEL
ERPOBDELLA SP	22310100	ERPO'LLA
ERPOBDELLA OCTOCULATA	22310101	ERPO.OCT
ERPOBDELLA TESTACEA	22310102	ERPO.TES
HYDRACARINA	24000000	HYDRACAR
CLADOCERA	29000000	CLADOCER
OSTRACODA	30000000	OSTRACOD
MYSIS RELICTA	35110101	MYSI.REL
ASELLUS SP	36110100	ASEL'LUS
ASELLUS AQUATICUS	36110101	ASEL.AQU
PROASELLUS MERIDIANUS	36110202	PROA.MER
CRANGONYX PSEUDOGRACILIS	37130101	CRAN.PSE
GAMMARUS SP	37140200	GAMM'RUS
GAMMARUS DUEBENI	37140202	GAMM.DUE
GAMMARUS LACUSTRIS	37140203	GAMM.LAC

GAMMARUS PULEX	37140206	GAMM.PUL
COLLEMBOLA	39000000	COLLEMBO
EPHEMEROPTERA	40000000	EPHRPTRA
SIPHLONURIDAE	40110000	SIPHLONU
SIPHLONURUS SP	40110100	SIPH'RUS
SIPHLONURUS ARMATUS	40110102	SIPH.ARM
SIPHLONURUS LACUSTRIS	40110103	SIPH.LAC
BAETIDAE	40120000	BAETIDAE
BAETIS RHODANI	40120107	BAET.RHO
CENTROPTILUM LUTEOLUM	40120201	CENT.LUT
CLOEON DIPTERUM	40120301	CLOE.DIP
CLOEON SIMILE	40120302	CLOE.SIM
PROCLOEON BIFIDUM	40120401	PROC.BIF
HEPTAGENIIDAE	40130000	HEPTAGEN
RHITHROGENA SEMICOLORATA	40130102	RHIT.SEM
HEPTAGENIA SP	40130200	HEPT'NIA
ECDYONURUS SP	40130400	ECDY'RUS
ECDYONURUS DISPAR	40130401	ECDY.DIS
ECDYONURUS TORRENTIS	40130403	ECDY.TOR
ELECTROGENA LATERALIS	40130502	ELEC.LAT
AMELETUS INOPINATUS	40140101	AMEL.INO
LEPTOPHLEBIIDAE	40210000	LEPTOPAE
LEPTOPHLEBIA SP	40210100	LEPT'BIA
LEPTOPHLEBIA MARGINATA	40210101	LEPT.MAR
LEPTOPHLEBIA VESPERTINA	40210102	LEPT.VES
PARALEPTOPHLEBIA SP	40210200	PARA'BIA
EPHEMERA SP	40320100	EPHE'ERA
EPHEMERA DANICA	40320101	EPHE.DAN
SERRATELLA IGNITA	40410201	SERA.IGN
CAENIS SP	40510200	CAEN'IS
CAENIS HORARIA	40510201	CAEN.HOR
CAENIS LUCTUOSA	40510202	CAEN.LUC
CAENIS RIVULORUM	40510204	CAEN.RIV
CAENIS ROBUSTA	40510205	CAEN.ROB
PLECOPTERA	41000000	PLECOPTE
RHABDIOPTERYX ACUMINATA	41110201	RHAB.ACU
NEMOURIDAE	41120000	NEMOURID
AMPHINEMURA SP	41120200	AMPH'URA
AMPHINEMURA SULCICOLLIS	41120202	AMPH.SUL
NEMURELLA PICTETI	41120301	NEMU.PIC
NEMOURA SP	41120400	NEMO'URA
NEMOURA AVICULARIS	41120401	NEMO.AVI
NEMOURA CAMBRICA	41120402	NEMO.CAM
NEMOURA CINEREA	41120403	NEMO.CIN
LEUCTRA SP	41130100	LEUC'TRA
LEUCTRA FUSCA	41130101	LEUC.FUS
LEUCTRA HIPPOPUS	41130103	LEUC.HIP
LEUCTRA INERMIS	41130104	LEUC.INE
LEUCTRA NIGRA	41130106	LEUC.NIG
CAPNIA SP	41140100	CAPN'IA
CAPNIA BIFRONS	41140102	CAPN.BIF
PERLODIDAE	41210000	PERLODID
PERLODES MICROCEPHALUS	41210201	PERL.MIC

DIURA BICAUDATA	41210301	DIUR.BIC
ISOPERLA GRAMMATICA	41210401	ISOP.GRA
CHLOROPERLA SP	41230100	CHLO'RLA
SIPHONOPERLA TORRENTIUM	41230301	SIPH.TOR
ZYGOPTERA	42100000	ZYGOPTER
COENAGRIONIDAE	42120000	COENAGRI
PYRRHOSOMA NYMPHULA	42120101	PYRR.NYM
ISCHNURA SP	42120200	ISCH'URA
ISCHNURA ELEGANS	42120201	ISCH.ELE
ENALLAGMA CYATHIGERUM	42120301	ENAL.CYA
COENAGRION PUELLA	42120405	COEN.PUE
ERYTHROMMA SP	42120600	ERYT'MMA
LESTES SPONSA	42130102	LEST.SPO
ANISOPTERA	42200000	ANISOPTE
CORDULEGASTRIDAE	42220000	CORDULEG
CORDULEGASTER BOLTONII	42220101	CORD.BOL
AESHNA SP	42230200	AESH'NA
CORDULIIDAE	42240000	CORDULII
CORDULIA SP	42240100	CORD'LIA
LIBELLULIDAE	42250000	LIBELLUL
SYMPETRUM SP	42250300	SYMP'RUM
SYMPETRUM NIGRESCENS	42250303	SYMP.NIG
VELIA (PLESIOVELIA) CAPRAI	43220111	VELI.CAP
GERRIS SP	43230100	GERR'IS
NOTONECTA SP	43510100	NOTO'CTA
NOTONECTA OBLIQUA	43510103	NOTO.OBL
CORIXIDAE	43610000	CORIXIDA
MICRONECTA SP	43610100	MICR'CTA
GLAENOCORISA PROPINQUA	43610401	GLAE.PRO
CALLICORIXA PRAEUSTA	43610501	CALL.PRA
CALLICORIXA WOLLASTONI	43610502	CALL.WOL
HESPEROCORIXA MOESTA	43610703	HESP.MOE
HESPEROCORIXA SAHLBERGI	43610704	HESP.SAH
ARCTOCORISA GERMARI	43610802	ARCT.GER
SIGARA SP	43610900	SIGA'RA
SIGARA (SIGARA) DORSALIS	43610911	SIGA.DOR
SIGARA (SUBSIGARA) DISTINCTA	43610921	SIGA.DIS
SIGARA (SUBSIGARA) SCOTTI	43610925	SIGA.SCO
PARACORIXA CONCINNA	43611101	PARA.CNC
COLEOPTERA	45000000	COLEOPTE
HALIPLIDAE	45110000	HALIPLID
HALIPLUS SP	45110300	HALI'LUS
HALIPLUS CONFINIS	45110302	HALI.CON
HALIPLUS FULVUS	45110305	HALI.FUL
HALIPLUS OBLIQUUS	45110314	HALI.OBL
HALIPLUS RUFICOLLIS	45110315	HALI.RUF
DYTISCIDAE	45140000	DYTISCID
HYGROTUS (HYGROTUS) QUINQUELINEATUS	45140613	HYGR.QUI
HYGROTUS (COELAMBUS) NOVEMLINEATUS	45140624	HYGR.NOV
HYDROPORUS SP	45140800	HYDP'RUS
HYDROPORUS FERRUGINEUS	45140807	HYDP.FER
HYDROPORUS MEMNONIUS	45140817	HYDP.MEM
HYDROPORUS PALUSTRIS	45140824	HYDP.PAL

DERONECTES SP	45141200	DERO'TES
NEBRIOPORUS SP	45141300	NEBR'POR
NEBRIOPORUS ASSIMILIS	45141301	NEBR.ASS
NEBRIOPORUS DEPRESSUS	45141303	NEBR.DEP
NEBRIOPORUS ELEGANS	45141305	NEBR.ELE
STICTOTARSUS DUODECIMPUSTULATUS	45141401	STIC.DUO
STICTOTARSUS MULTILINEATUS	45141402	STIC.MUL
OREODYTES SP	45141500	OREO'TES
OREODYTES DAVISII	45141501	OREO.DAV
OREODYTES SANMARKII	45141502	OREO.SAN
OREODYTES SEPTENTRIONALIS	45141503	OREO.SEP
AGABUS ARCTICUS	45142002	AGAB.ARC
AGABUS BIPUSTULATUS	45142004	AGAB.BIP
ILYBIUS FULIGINOSUS	45142104	ILYB.FUL
RHANTUS BISTRIATUS	45142202	RHAN.BIS
RHANTUS EXSOLETUS	45142203	RHAN.EXS
GYRINIDAE	45150000	GYRINIDA
GYRINUS SP	45150200	GYRI'NUS
GYRINUS AERATUS	45150201	GYRI.AER
ORECTOCHILUS VILLOSUS	45150401	OREC.VIL
HYDROPHILIDAE	45350000	HYDROPHL
PARACYMUS AENEUS	45351001	PARA.AEN
ANACAENA GLOBULUS	45351302	ANAC.GLO
HYDRAENA SP	45410200	HYDR'ENA
HYDRAENA GRACILIS	45410202	HYDR.GRS
DRYOPS SP	45620200	DRYO'PS
ELMIDAE	45630000	ELMIDIDA
ELMIS SP	45630100	ELMI'S
ELMIS AENEA	45630101	ELMI.AEN
ESOLUS PARALLELEPIPEDUS	45630201	ESOL.PAR
LIMNIUS VOLCKMARI	45630301	LIMN.VOL
OULIMNIUS SP	45630600	OULI'IUS
OULIMNIUS TROGLODYTES	45630603	OULI.TRO
OULIMNIUS TUBERCULATUS	45630604	OULI.TUB
SIALIS SP	46110100	SIAL'IS
SIALIS LUTARIA	46110102	SIAL.LUT
TRICHOPTERA	48000000	TRICHOPT
AGAPETUS SP	48120200	AGAP'TUS
AGRAYLEA MULTIPUNCTATA	48130101	AGRA.MUL
HYDROPTILA SP	48130300	HYDR'ILA
OXYETHIRA SP	48130400	OXYE'IRA
PSYCHOMYIIDAE	48220000	PSYCHOMY
TINODES SP	48220400	TINO'DES
TINODES WAENERI	48220408	TINO.WAE
POLYCENTROPODIDAE	48240000	POLYCENT
CYRNUS SP	48240100	CYRN'US
CYRNUS FLAVIDUS	48240101	CYRN.FLA
CYRNUS INSOLUTUS	48240102	CYRN.INS
CYRNUS TRIMACULATUS	48240103	CYRN.TRI
PLECTROCNEMIA SP	48240400	PLEC'MIA
PLECTROCNEMIA CONSPERSA	48240402	PLEC.CON
PLECTROCNEMIA GENICULATA	48240403	PLEC.GEN
POLYCENTROPUS SP	48240500	POLY'PUS

POLYCENTROPUS FLAVOMACULATUS	48240501	POLY.FLA
POLYCENTROPUS KINGI	48240503	POLY.KIN
HYDROPSYCHE SILTALAI	48250209	HYDR.SIL
AGRYPNIA SP	48310100	AGRY'NIA
AGRYPNIA OBSOLETA	48310102	AGRY.OBS
AGRYPNIA PICTA	48310104	AGRY.PIC
AGRYPNIA VARIA	48310105	AGRY.VAR
PHRYGANEA GRANDIS	48310502	PHRY.GRA
BRACHYCENTRUS SUBNUBILUS	48320101	BRAC.SUB
LEPIDOSTOMATIDAE	48330000	LEPIDOST
LEPIDOSTOMA HIRTUM	48330301	LEPI.HIR
LIMNEPHILIDAE	48340000	LIMNEPAE
IRONOQUIA DUBIA	48340101	IRON.DUB
HALESUS SP	48340600	HALE'SUS
HALESUS DIGITATUS	48340601	HALE.DIG
HALESUS RADIATUS	48340602	HALE.RAD
MESOPHYLAX IMPUNCTATUS	48340902	MESO.IMP
MICROPTERNA SP	48341000	MICR'RNA
POTAMOPHYLAX CINGULATUS	48341101	POTA.CIN
POTAMOPHYLAX LATIPENNIS	48341102	POTA.LAT
CHAETOPTERYX VILLOSA	48341301	CHAE.VIL
ANABOLIA NERVOSA	48341401	ANAB.NER
LIMNEPHILUS SP	48341700	LIMN'LUS
LIMNEPHILUS AURICULA	48341702	LIMN.AUR
LIMNEPHILUS CENTRALIS	48341706	LIMN.CEN
LIMNEPHILUS LUNATUS	48341719	LIMN.LUN
LIMNEPHILUS MARMORATUS	48341722	LIMN.MAR
LIMNEPHILUS POLITUS	48341725	LIMN.POL
LIMNEPHILUS RHOMBICUS	48341726	LIMN.RHO
LIMNEPHILUS SUBCENTRALIS	48341729	LIMN.SUC
LIMNEPHILUS VITTATUS	48341732	LIMN.VIT
GOERA PILOSA	48350101	GOER.PIL
ERNODES ARTICULARIS	48360301	ERNO.ART
SERICOSTOMA PERSONATUM	48370201	SERI.PER
DIPTERA	50000000	DIPTERA
TRICLADIDA	50000000	TRICLADI
TIPULIDAE	50110000	TIPULDAE
TIPULA SP	50110300	TIPU'LA
LIMONIIDAE	50130000	LIMONIID
ANTOCHA SP	50130500	ANTO'CHA
HELIUS SP	50130900	HELI'US
LIMNOPHILA SP	50132000	LIMN'ILA
PEDICIIDAE	50140000	PEDICIID
DICRANOTA SP	50140500	DICR'OTA
PERICOMA SP	50210200	PERI'OMA
CHAOBORIDAE	50320000	CHAOBORI
CULICIDAE	50330000	CULICIDA
CERATOPOGONIDAE	50350000	CERATOPO
SIMULIIDAE	50360000	SIMULIID
CULICOIDEA (CHIRONOMIDAE)	50400000	CHIRODAE
TABANOIDEA	50600000	TABANOID
TABANIDAE	50630000	TABANIDA
EMPIDIDAE	50710000	EMPIDIDA

HEMERODROMIA SP	50711300	HEME'MIA
WIEDEMANNIA SP	50712000	WIED'NIA
LIMNOPHORA SP	50850200	LIMN'ORA
PHOXINUS PHOXINUS	52080901	PHOX.PHO
GASTEROSTEUS ACULEATUS	52130101	GAST.ACU
TRITURUS SP	53110100	TRIT'RUS
LEPTOCERIDAE	483A0000	LEPTOCER
ATHRIPSODES SP	483A0100	ATHR'DES
ATHRIPSODES ATERRIMUS	483A0102	ATHR.ATE
ATHRIPSODES BILINEATUS	483A0103	ATHR.BIL
ATHRIPSODES CINEREUS	483A0104	ATHR.CIN
CERACLEA ANNULICORNIS	483A0202	CERA.ANN
MYSTACIDES SP	483A0400	MYST'DES
MYSTACIDES AZUREA	483A0401	MYST.AZU
MYSTACIDES LONGICORNIS	483A0402	MYST.LON
TRIAENODES BICOLOR	483A0701	TRIA.BIC
OECETIS SP	483A0900	OECE'TIS
OECETIS OCHRACEA	483A0904	OECE.OCH
OECETIS TESTACEA	483A0905	OECE.TES
SETODES ARGENTIPUNCTELLUS	483A1001	SETO.ARG