Developing typology and class boundaries for WFD-AWICsp to assess acidification in UK rivers using macroinvertebrates

Report to the UKTAG Freshwater Task Team

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Author: Ben McFarland

This report documents work undertaken by the Freshwater Task Team of the WFD UK Technical Advisory Group, supporting the development of a tool (WFD-AWIC) to describe ecological status of macroinvertebrate communities in rivers affected by acidification. The report is provided as supporting information, details of the application of the tool are available in the UKTAG Method Statement, published on the UKTAG website (www.wfduk.org).

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Executive Summary

The acidity sensitive metric, AWICsp, has been updated for the purposes of the Water Framework Directive (WFD). Abundance is now included and the new metric is known as WFD-AWICsp. Testing using linear regression improved explanatory power compared to AWICsp and AWICF.

Reference sites were chosen using chemical and biological screening. The chemical screening used Acid Neutralising Capacity (ANC) and pH thresholds in relation to the level of calcium. pH was excluded if a site had Dissolved Organic Carbon (DOC) >10 mg/l, to account for low pH at sites with high levels of naturally occurring humic acids. Biological screening removed sites if a sample did not contain a certain number of specified sensitive taxa. Sites had to pass both screening stages to be included as reference.

Testing using ANOSIM revealed significant differences in community structure between Welsh and Scottish references sites. Differences were also found at Scottish sites >10 mg/l DOC. These results suggest a different typology should be used between Wales and Scotland. In Scotland a further type should occur at high DOC sites (>10 mg/l).

A SIMPER test was performed to determine which taxa contribute to similarity within-type. These taxa were then used to construct an expected reference community for each type and derive expected WFD-AWICsp scores.

Boundaries were identified from step-changes in pressure-response using Lowess Smoothing. Structural, diversity and functional metrics were used to assess community response. These thresholds were then used to predict EQR values using linear regression between pressure (ANC) and WFD-AWICsp, enabling the identification of boundary values. Sites were then classified using these boundaries. Contour plots enabled a evaluation of EQR and status class in relation to ANC and pH.

All classified sites were then validated using multivariate analysis by using different aspects of structural and functional community composition. Results indicated a continuum of change across the classes, although there were examples of distinct differences between classes in some cases. On most occasions, sites placed in an apparently ‘incorrect’ area of the ordination or cluster, based on their status, could be explained by the presence (or absence) of sensitive taxa. This suggests that WFD-AWICsp classification, not only reflects community change in structure, function and diversity, but is also robust in correctly classifying more atypical sites.

No boundaries have been developed explicitly for English sites. This was due to the lack of data to the required taxonomic level. The few sites that were from England were closest structurally and functionally to Welsh sites. It is therefore recommended that English sites are compared to the expected community for Wales and compared to the Welsh boundaries.

It is recommended chemical sampling is taken on a monthly basis to increase the chance of capturing episodic acid events. The relatively high Acid Neutralising Capacity (ANC) values at which community change was noted, suggests many of these events have been missed. Chemical data that are required to support the classification are Cantrell ANC (Cantrell, 1990), pH and Dissolved Organic Carbon (DOC). The inclusion of inorganic monomeric aluminium within Cantrell ANC will improve the accuracy of results.

1.0 Background to the development of WFD-AWICsp

1.1 Development of the final AWICsp

Following the Re-testing report (McFarland, 2009), John Murphy agreed to re-look at AWICsp and review the taxa within the metric and their respective scores. The detailed results are contained in the report, 'Revision of AWICsp biotic index' (Murphy, 2009). This report accepted the general comments regarding;

1) Concern over the use of taxon groups higher than genus. For example, the family Chironomidae has many species whose optima are likely to be spread along the pressure gradient.

2) Concern over the sensitivity classes assigned to some species (see Rendall *et al*, 2009).

3) Results from the Re-testing report (McFarland, 2009). This report suggested that many of the species of concern identified in Rendall *et al* (2009) may have been ascribed to an incorrect sensitivity class.

4) The use of data from sites > pH 6.8. The use of circumneutral sites may erroneously influence the location of the pH optima of a taxon within the Canonical Correspondence Analysis (CCA).

5) The lack of Scottish data.

The resulting AWICsp metric removed taxa groups above genus, only used data from sites with pH <6.8 and included Scottish data within the CCA. Table 1, taken from Murphy (2009), shows the final list of taxa within AWICsp.

Table 1: Taxa used in the final AWICsp (Murphy, 2009)

CODE	Name	AWICsp score	CCA1 score	% Dist
48120200	*Agapetus* sp.	9	-0.9165	100
40510200	*Caenis* sp.	9	-0.8865	99
40120105	*Baetis muticus*	9	-0.874	98
48120100	*Glossosoma* sp.	9	-0.867	98
16140301	*Potamopyrgus jenkinsi*	9	-0.7933	94
37140206	*Gammarus pulex*	9	-0.7902	94
41220201	*Perla bipunctata*	9	-0.7508	92
16240101	*Ancylus fluviatilis*	9	-0.6888	90
48210101	*Philopotamus montanus*	8	-0.6799	89
48350202	*Silo pallipes*	8	-0.6688	89
48210200	*Wormaldia* sp.	8	-0.6615	88
48250206	*Hydropsyche instabilis*	8	-0.6558	88
40130400	*Ecdyonurus* sp.	8	-0.5792	85
40130100	*Rhithrogena* sp.	8	-0.5678	84
45410202	*Hydraena gracilis*	8	-0.5469	83
48370201	*Sericostoma personatum*	8	-0.502	81
40130204	*Heptagenia sulphurea*	8	-0.4973	81
50620100	*Atherix* sp.	8	-0.4902	81
45630201	*Esolus parallelepipedus*	8	-0.4828	80
40120107	*Baetis rhodani*	8	-0.4826	80
41210201	*Perlodes microcephala*	7	-0.4465	79
48330301	*Lepidostoma hirtum*	7	-0.3969	76
48250301	*Diplectrona felix*	7	-0.3385	74
40130202	*Heptagenia lateralis*	7	-0.318	73
48250209	*Hydropsyche siltalai*	6	-0.2215	68
48250207	*Hydropsyche pellucidula*	6	-0.2179	68
40120106	*Baetis niger*	6	-0.1855	67
45630101	*Elmis aenea*	6	-0.1549	65
41230103	*Chloroperla tripunctata*	6	-0.1509	65
45630301	*Limnius volckmari*	6	-0.1361	64
05110401	*Crenobia alpina*	6	-0.0642	61
42220101	*Cordulegaster boltonii*	5	0.0084	58
41210401	*Isoperla grammatica*	5	0.0163	57
41110302	*Brachyptera risi*	5	0.0887	54
48110101	*Rhyacophila dorsalis*	5	0.1765	50
05110301	*Phagocata vitta*	4	0.2001	49
41230102	*Chloroperla torrentium*	4	0.22	48
41130104	*Leuctra inermis*	4	0.2317	48
45630600	*Oulimnius* sp.	4	0.2485	47
41120202	*Amphinemura sulcicollis*	4	0.2799	45
41120100	*Protonemura* sp.	4	0.2875	45
41130106	*Leuctra nigra*	4	0.3829	41
41130103	*Leuctra hippopus*	3	0.4974	36
40210101	*Leptophlebia marginata*	3	0.5222	34
46110100	*Sialis* sp.	3	0.5578	33
41210301	*Diura bicaudata*	3	0.5727	32
41120400	*Nemoura* sp.	2	0.7676	23
41120301	*Nemurella picteti*	1	1.2782	0

1.2 Development of a WFD compliant metric

To make the new AWICsp compliant for the WFD, abundance should be considered. To do this WFD-AWICsp was developed, incorporating abundance based on the log scale commonly used within the agencies. Whilst many labs now record estimated actual abundance, the use of a log scale has the advantage of enabling WFD-AWICsp to be used on historic data, where mixed-taxon samples are available. The abundance weighting used is shown in Table 2. This is based on a matrix design similar to those used in many other metrics e.g. LIFE (Extence *et al*, 1999) whereby sensitive taxa score more with increasing abundance, whereas tolerant taxa score less.

Table 2: Scores for different log abundance categories in each sensitivity class.

Log Abundance counts	**Highly Tolerant**	**Tolerant**	**Moderately Tolerant**	**Moderately Sensitive**	**Sensitive**	**Highly Sensitive**
A or 1 (1-9)	**3**	**5**	**7**	**8**	**10**	**12**
B or 2 (10-99)	**2**	**4**	**6**	**9**	**11**	**13**
C+ or 3+ (100-999)	**1**	**3**	**5**	**10**	**12**	**14**

Sensitivity classes were assigned by firstly splitting the list into broad groups of sensitive and tolerant taxa based on their position in relation to the CCA centriod. Taxa with negative scores on the CCA axis were assigned to sensitive classes, species with positive scores were assigned to tolerant classes. These were then split further into six sensitivity classes (Table 3). More than six classes result in the matrix becoming unwieldy, less than six result in the increased potential loss of precision by over-simplifying the original AWICsp scores.

Table 3: AWICsp scores split by sensitivity class

AWICsp Score	Sensitivity Class
9	**Highly Sensitive**
8	**Sensitive**
7	**Moderately Sensitive**
6	**Moderately Sensitive**
5	**Moderately Tolerant**
4	**Tolerant**
3	**Tolerant**

2	**Highly Tolerant**
1	**Highly Tolerant**

1.3 Testing of WFD-AWICsp

The performance of the new WFD-AWICsp metric was determined by testing against a dataset (N=49), independent from the data used to derive the species scores. The dataset contained sites across the pH and ANC ranges (4.24-6.8 and -20.15-140.53 µeq/l respectively). Chemical data were included if sampled from the preceding two years from the date of the biological sample. Table 4 indicates that the addition of abundance (WFD-AWICsp) explains slightly more variability than presence/absence (AWICsp). The modest increase in explanatory power may be due to the low numbers of positively responding tolerant taxa. This contrasts to organic pollution where a wide range of species can increase with organic enrichment. Nevertheless, given that the assessment of abundance is mentioned in the normative definitions of the WFD, it is proposed the weighting within Table 2 is retained for classification. It is interesting to note that both metrics appear to respond to pressure noticeably more than the family-based metric, AWICF (Davy-Bowker *et al*, 2005). In this case the species metrics fit the data better (and can more accurately predict pressure values), although this does not explicitly link causality between pressure and response. Despite this, the results do lend support to the use of species level data and abundance in assessing river acidification.

Table 4: Results from testing AWICsp, WFD-AWICsp and AWICF on an independent dataset using linear regression (where *P*-value; *** = <0.001, ** = <0.01, * = <0.05) after testing for normality using Ryan-Joiner, Johnson's transformation where appropriate and Bonferroni correction (significance at *P* = <0.008).

Metric	Pressure	N	R^2	*P*-value
WFD-AWICsp	ANC	49	0.61	***
AWICsp	ANC	49	0.59	***
AWICF	ANC	49	0.33	**
WFD-AWICsp	pH	49	0.48	***
AWICsp	pH	49	0.46	***
AWICF	pH	49	0.19	*

The results of the regressions for WFD-AWICsp are shown in Figures 1 and 2 for ANC and pH respectively.

An interesting observation is the stronger relationship between the metrics and ANC compared to pH using these data. The additional unexplained variance for the pH regressions might be explained by the number of high humic sites within the dataset. Below pH 5.5 there are nine sites above the regression line, of which eight are humic (>10 mg/l DOC) (Figure 2). The clear-water site still has a comparatively high DOC (>8 mg/l). This supports previous work (e.g. McFarland *et al*, 2009; Dangles *et al,* 2004) that low pH caused by humic acids can still support a diverse number of sensitive taxa. Only when sites with low pH at high DOC levels, have correspondingly low ANC, does it become more certain a site is acidified, rather than naturally acidic. This is illustrated by the spread of data for low pH, humic sites, along the ANC gradient, despite all sites having pH <5.5 (Figure 1). Humic sites below the regression line for pH and ANC are more likely to be sites that are acidified.

Figure 1: Linear regression of WFD-AWICsp verses ANC (μeq/l). Humic sites (>10 mg/l DOC) below pH 5.5 are represented by ♦

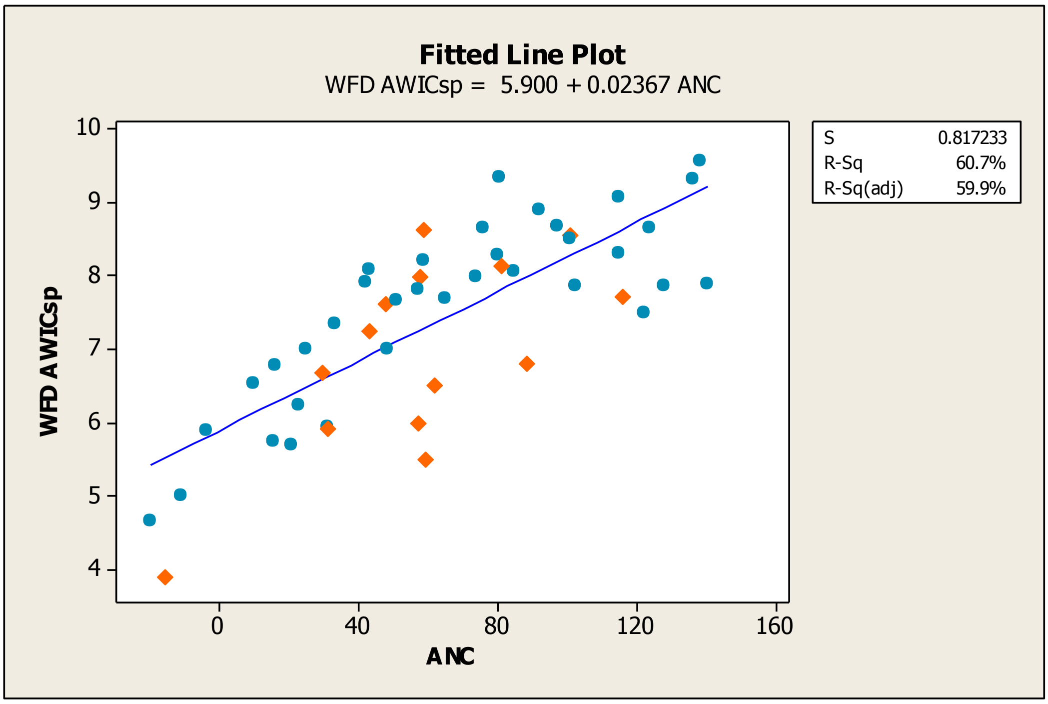
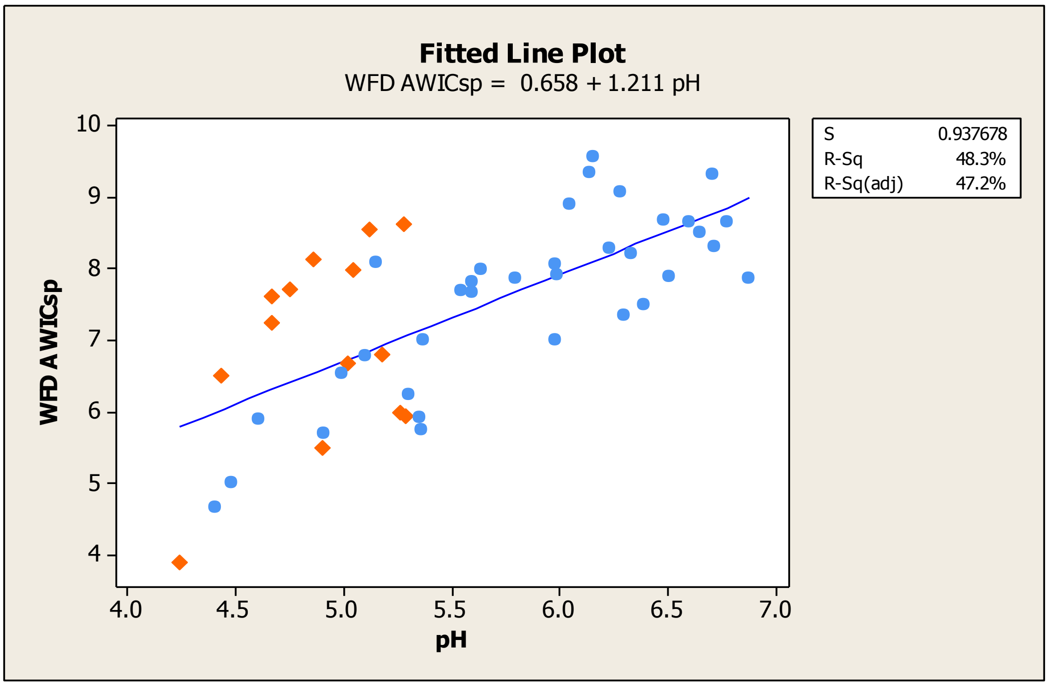


Figure 2: Linear regression of WFD-AWICsp verses pH. Humic sites (>10 mg/l DOC) below pH 5.5 are represented by ♦


Fitted Line Plot
WFD AWICsp = 0.658 + 1.211 pH
S 0.937678
R-Sq 48.3%
R-Sq(adj) 47.2%
WFD AWICsp
pH
4.0
4.5
5.0
5.5
6.0
6.5
7.0
4
5
6
7
8
9
10

2.0 Reference site screening

The reference site screening was done on all data used in the previous reports. Only sites with at least four chemical samples within two years prior to the biological sample were used. For sites (SEPA, EA and FRS data) to be included they had to have the mean of the lowest two values below ANC 150 µeq/l and pH 7 and a mean Ca below 4 mg/l. For the Welsh Acid Water (WAWS) data, mean values were used, derived from winter means. Kowalik data used measurements gathered during high flow events. Individual sample values were not available for either of these datasets. This approach attempts to capture episodic events during periods where acidification pressure is likely to be most acute. This ensured only sites which had the potential to be acidified were included, providing a more meaningful representative reference fauna. As with other reports, the biological data had to be to mixed-taxon level ensuring reliable calculations of WFD-AWICsp. A total of 218 sites passed this initial screening from the following datasets;

WAWS – 88 sites

Kowalik PhD – 72 sites

FRS – 31 sites

SEPA – 25 sites

EA – 2 sites

The actual screening to assess reference used a two-stage process. The first stage was a chemical screening stage. Sites that passed this screening then went on to the second stage using a biological screening criteria.

2.1 Chemical screening

The role of DOC in acid waters can be seen in the different relationships of pH and ANC to the DOC gradient (Figure 3). This supports the view that DOC and naturally occurring humic acids can have a substantial impact on pH. Care must be taken, therefore, when screening sites for use as reference, as streams can have naturally low pH when DOC is high. Numerous studies have indicated that these naturally occurring brown water, acidic sites have their own reference communities, where apparently acid sensitive species and fully functioning communities can occur at low pH (e.g. Moe *et al.*, 2009; Petrin *et al.*, 2008; Dangles *et al.,* 2004). Any screening for reference (and typology development) needs to consider these naturally acid sites. Without consideration for DOC levels many naturally acidic reference sites are likely to be removed.

The first stage of the reference site screening used the DAM typology approach (Table 5) (Kelly, 2005). Figure 4 suggests the effect of DOC on declining pH only becomes significant once DOC levels rise above 10 mg/l and therefore pH is ignored in the screening above this threshold. Above 10 mg/l only ANC levels in relation to Ca have been used, providing a more reliable assessment for brown water acidic sites.

Table 5:Chemical screening for reference sites based on the DAM typology (Kelly, 2005).

Type	Ca (μeq/l)	Reference	
		pH*	ANC (μeq/l)
5 (extremely low Ca)	<50	>5.6	>20
6 (very low Ca)	≥50;<100	>6.0	>50
7 (low ca)	≥100;<300**	>6.2	>50

*Only used when DOC <10 mg/l

**Only sites with Ca <4 mg/l included as potential reference sites. This equates to approx 200 μeq/l

Figure 3: Scatterplot with linear regression line illustrating the effect DOC on ANC (Left) and pH (right) (using sites passing the DAM reference screening).

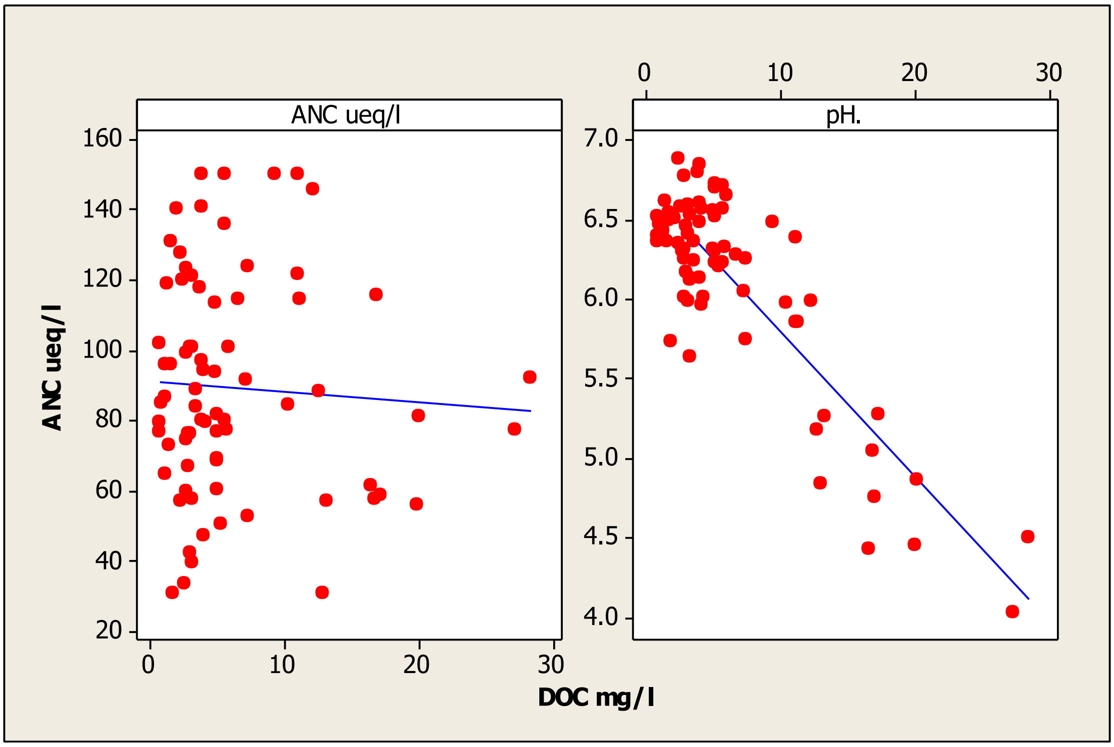
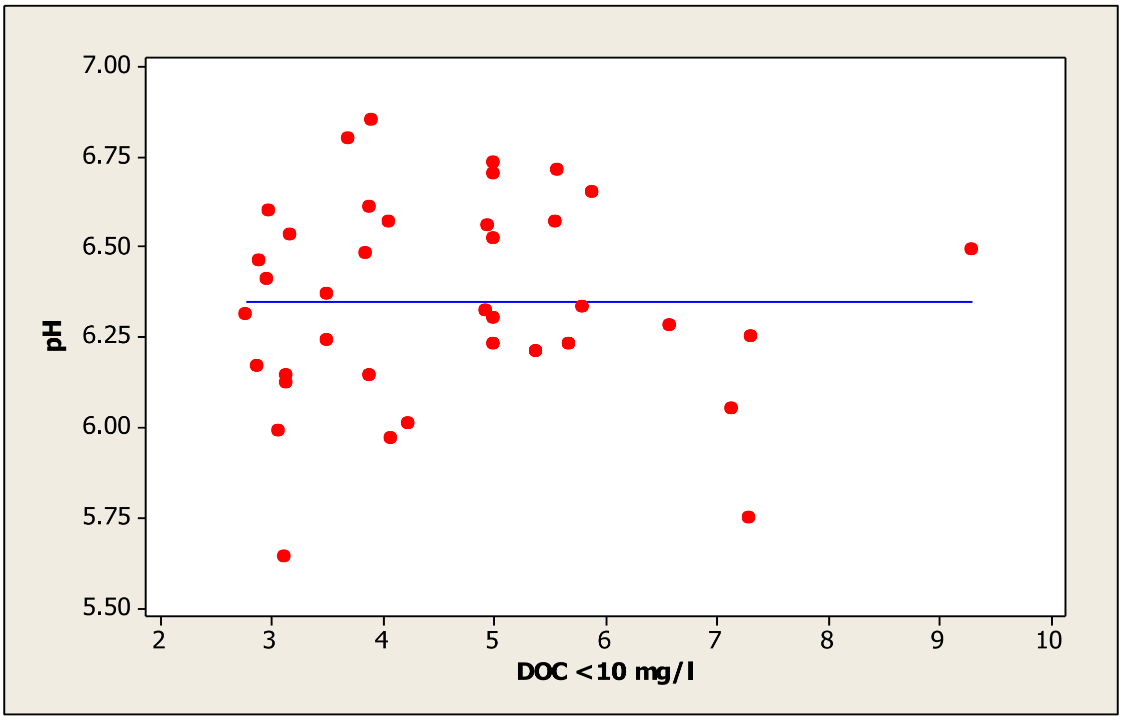


Figure 4: Scatterplot with linear regression line illustrating the effect DOC on pH at sites <10 mg/l DOC (using sites passing the DAM reference screening).



2.2 Biological Screening

The second stage utilised biological data. For a site to pass, the biological sample had to contain key sensitive taxa following Ormerod and Durance (2009) (Table 6). Each sample had to either have a minimum of 3 taxa, including at least 2 in group IV, or total of 5 from either groups. This second stage helped to screen out sites that suffer from episodic acid events which might have been missed during chemical sampling.

The process of chemical and biological screening resulted in 45 reference sites (Table 7).

Table 6: Invertebrate taxa characterised by mean and minimum pH (Ormerod & Durance, 2009)

Group III	**Group IV**
Mean pH 6.1-6.4	**Mean pH 6.4-6.7**
Minimum pH 4.9-5.7	**Minimum pH > 5.7**
Oreodytes sanmarkii	*Silo pallipes*
Tabanidae	*Ancylus fluviatilis*
Crenobia alpina	*Baetis rhodani gp*
Sialis sp	*Caenis sp*
Perlodes microcephalus	*Wormaldia sp*
Pisidium sp	*Perla bipunctata*
Ceratopogonidae	*Glossossoma conformis*
Lepidostoma hirtum	*Metalype fragilis*
Hydraena gracilis	*Odontocerum albicorne*
Helichus substriatus	*Alainites muticus*
Sericostoma personatum	*Serratella ignita*
Diplectrona felix	*Agapetus fuscipes*
Rhithrogena semicolorata	*Limnebius truncatellus*
Ecdyonurus sp	*Crunoecia irrorata*
Electrogena lateralis	Lymnaeidae
Hydropsyche instabilis	Dixidae
Philopotamus montanus	*Paraleptophlebia submarginata*
	Helophorus sp

Table 7: Reference Sites after screening process, with relevant chemical and biological data. Sites sorted by level of DOC.

Site (data source)	Region	pH	ANC (μeq/L)	Ca (μeq/L)	DOC mg/l	Group III Taxa	Group IV Taxa
Ceiliog (WAWS)	Wales	6.52	101.9	64.34	0.68	3	4
Berwyn (WAWS)	Wales	6.37	79.26	143.64	0.73	4	1
Iar (WAWS)	Wales	6.4	77	49.88	0.78	3	3
Ceredig (WAWS)	Wales	6.47	85.22	71.32	0.87	5	6
LI6 (Kowalik)	Wales	6.44	86.47	87.65	1.2	3	3
Wnion (WAWS)	Wales	6.43	64.93	121.1	1.21	5	1
Hust (WAWS)	Wales	6.62	118.91	159.6	1.32	5	8
Cynhenfoed (WAWS)	Wales	6.55	131.1	183.04	1.56	6	2
Llwydcoed (WAWS)	Wales	6.5	95.7	197.51	1.62	2	2
Slugain (FRS)	Scotland	6.88	128	138.67	2.27	2	3
Helygog (WAWS)	Wales	6.35	56.84	89.28	2.28	4	1
Allt Mharcaidh (SEPA)	Scotland	6.3	33.25	35.67	2.54	2	2
R. Feshie (SEPA)	Scotland	6.78	123.6	111.52	2.69	2	3
NW17 (Kowalik)	Scotland	6.01	59.62	34.16	2.77	3	2
Llugwy (WAWS)**	Wales	6.31	74.39	80.8	2.77	6	1
Ceirw (WAWS)**	Wales	6.46	76.19	137.66	2.89	7	1
Dyfrdwy (WAWS)	Wales	6.41	100.74	140.15	2.96	5	1
R. Ewe (SEPA)	Scotland	6.6	76.03	61.55	2.99	2	4
Etive, Mheuran (SEPA)	Scotland	5.99	42.15	47.36	3.07	2	2
Groes (WAWS)	Wales	6.53	121.37	168.08	3.18	4	2
Brefi (WAWS)	Wales	6.37	83.95	112.22	3.51	5	2
GA27 (Kowalik)	Scotland	6.8	118.1	107.77	3.7	2	2
R.Scaddle (SEPA)	Scotland	6.48	97.15	76.74	3.85	3	2
R. Gruinard (SEPA)	Scotland	6.14	80.28	49.83	3.89	3	4
GA36 (Kowalik)	Scotland	6.57	94.27	78.63	4.06	3	4
NW19 (Kowalik)	Scotland	5.97	47.23	37.2	4.08	3	2
Water of Feugh (SEPA)	Scotland	6.01	79.73	60.35	4.24	3	2
Abhainn Inverb (SEPA)	Scotland	6.32	93.73	58.64	4.93	3	3
NW32 (Kowalik)	Scotland	6.56	113.3	72.16	4.95	3	2
Hesgin (EA)	Wales	6.3	69.1	197.04	<5.0	2	2
Tame (EA)	England	6.7	68.82	151.89	<5.0	3	2
Callater Burn (SEPA)	Scotland	6.71	136.14	>100	5.58	3	3
Allt Bad an Luig (SEPA)	Scotland	6.33	77.1	80.01	5.8	5	5
Baddoch Burn (SEPA)	Scotland	6.28	114.75	>100	6.58	2	3
River Carron (SEPA)	Scotland	6.05	91.75	87.39	7.14	2	3
River Hope (SEPA)	Scotland	6.25	123.95	59.19	7.32	2	3
R Borgie SEPA)	Scotland	6.49	148.22	153.46	9.29	3	5
Big Water Fleet (SEPA)	Scotland	5.86*	149.1	140.27	11.02	4	2
Carie Burn (SEPA)	Scotland	6.39*	121.95	162.24	11.09	2	3
Achridigill (FRS)	Scotland	5.26*	57.25	89	13.15	3	2
Forsinain (FRS)	Scotland	5.04*	57.63	121.5	16.74	4	3
Thurso River (SEPA)	Scotland	4.75*	115.68	186.49	16.88	4	3
River Tirry (SEPA)	Scotland	5.28*	58.67	131.15	17.26	4	4
Feoch Burn (SEPA)	Scotland	4.45*	56.05	103.09	19.99	5	0
Tarf Water (SEPA)	Scotland	4.86*	81.25	140.57	20.09	6	1

* pH not used in screening due to DOC >10 mg/l

** Not included in analysis using PRIMER due to log abundance data only

3.0 Investigating and developing an ecologically relevant typology

All statistical tests in this section were performed in PRIMER 6.

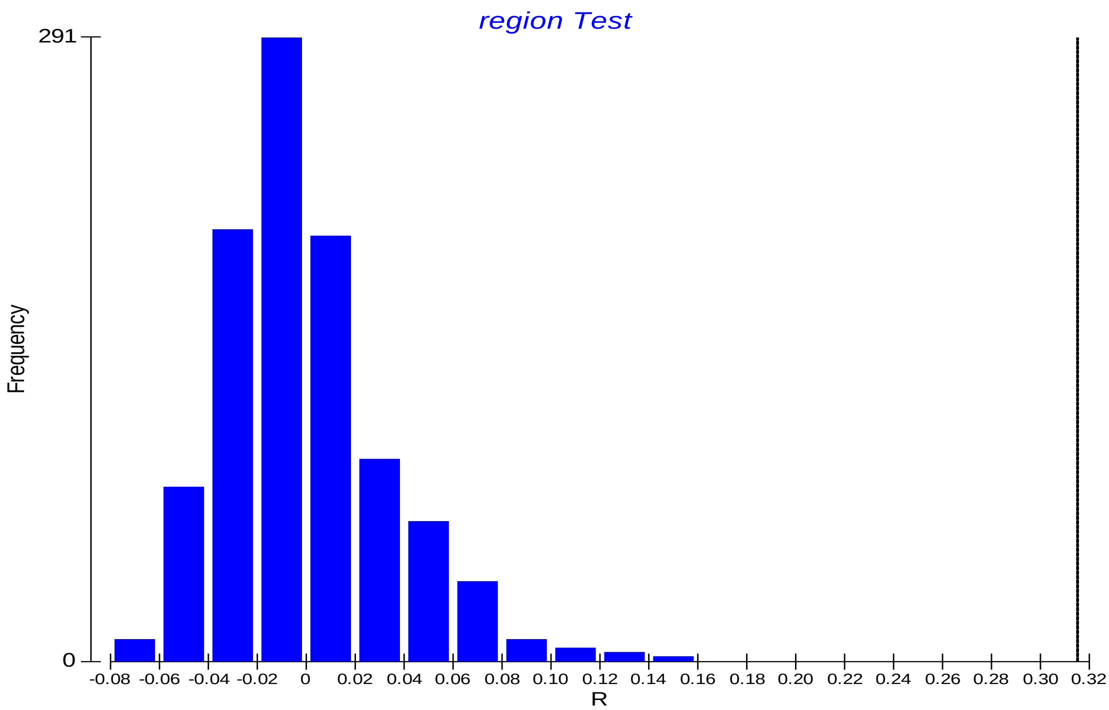
3.1 Testing for *a priori* differences

To investigate further whether the results from the regression shown in Figures 1 and 2 are reflected in a genuinely different reference fauna (i.e. a different community over 10 mg/l DOC), and to assess other potential differences, ANOSIM (analysis of similarity) was used to test for *a priori* differences in the structure of the reference community. This approach can be viewed as a non-parametric version of a multivariate ANOVA (MANOVA).

3.1.1 Testing between regions

Firstly a one-way ANOSIM was carried out on a fourth-root transformed Bray-Curtis matrix of all reference sites, with 999 permutations, using region as a factor. This was to test for possible differences in reference community between regions (i.e. Wales and Scotland, the single site from England was removed). The result suggested there are significant differences between the two sets of data (Figure 5, Global R = 0.315, p = 0.001). This implies a different set of reference values are required for Scotland and Wales.

Figure 5: One-Way ANOSIM testing for differences between reference communities in Wales and Scotland. The figure shows that the Global R (denoted by the vertical bold line) is greater than any of the 999 simulated permutations, rejecting the null hypothesis that there are no differences between the two communities.



3.1.2 Testing between types

A one-way ANOSIM was then carried out on the same matrix, this time with regions split into typology based on the DAM typology to determine the effect of varying levels of calcium. A further split was made at 10 mg/l DOC based on the findings that above this figure pH can fall dramatically and this might drive community change, despite high ANC.

This process results in the following groups;

Group A – Humic-water sites (>10 mg/l DOC).

Group B – Clear-water sites in DAM type 5 (<50 µeq/l Ca)

Group C - Clear-water sites in DAM type 6 (≥50;<100 µeq/l Ca)

Group D - Clear-water sites in DAM type 7 (≥100;<200 µeq/l Ca)

The global R was found to be significant (Global R = 0.38 p = 0.001) permitting further investigation of the pair-wise tests. Results of all the pair-wise tests can be seen in Table 8. The results support the hypothesis that there are significant differences in the composition of the reference community between Wales and Scotland with most pair-wise comparisons producing significant results. Of the twelve possible combinations only two are not significant. One of these (Wales B v Scotland B) had a very low number of permutations (N=10), making interpretation difficult. The second combination (Wales C v Scotland B) was only marginal (p = 0.06), with only five out of the 84 permutations being greater than the observed R. Whilst the p-value is >0.05, the threshold commonly taken to imply significance, there were only relatively few permutations in the pair-wise test. The R value is largely not a function of N, wheras p is always affected by sample size and so on this occasion the high R (0.469) may infer a significant difference.

In Scotland, sites in group A (i.e. humic rivers) are different to other groups in five of the six pair-wise comparisons, strongly supporting the need for a typology at naturally high humic, low pH sites. Sites from group A are significantly different to all Welsh groups and groups D and C in Scotland. However, there is no difference with group B. This may suggest that in Scotland reference sites with very low levels of calcium have communities indistinct from those found in humic waters. Despite this, whilst the p-value is >0.05, it is still low (0.09), reflecting the low number of permutations that are greater than the observed R (15 out of 165). Furthermore, group B is not significantly different to either group C or D, in contrast to group A. This suggests DOC, rather than calcium, is driving community structure at reference sites.

For the Welsh data there were no significant differences between DAM types suggesting calcium has limited influence in driving community, at least at levels <4 mg/l. This supports the findings from Scotland where the only clear significant differences between groups are when DOC over 10 mg/l is used as a threshold. None of the Welsh sites had a mean DOC >10 mg/l and so a similar pattern could not be tested for. Further, the influence of region, rather than calcium, can be seen in the difference between two of the three groups in common between both regions (C v C and D v D). The third (B v B) only had 10 possible permutations. Despite these findings, it seems likely calcium will have a bigger influence in influencing the community if higher calcium sites had been included.

These results suggest that a different set of reference sites must be used between Wales and Scotland. In Scotland it appears a further typology should occur at levels of high DOC. In Wales, there is no evidence that different types occur, suggesting a Welsh typology can be used. It must be remembered that the database itself is from a very restricted subset of samples from each region. In otherwords, the initial screening for sites to be included (pH <7.0, ANC <150 µeq/l and Ca <4mg/l) (i.e. only including those sites that might be at risk from acidification) creates a quite distinct typology in itself, where we might reasonably expect the faunal communities to be very similar between different water bodies. Therefore having one single type for rivers at risk from acidification in Wales is not unrealistic.

Table 8: Results of one-way ANOSIM pair-wise tests. Groups based on region (Scotland and Wales) and then split further by; 1) DAM typology for clear water types (<10 mg/l DOC) (Groups B, C and D) , to determine the effect of varying levels of calcium and 2) Humic water sites (>10 mg/l DOC) (Group A).

Groups	Observed R	P	Permutations	Number >= Observed R
Cwal, Dwal	0.047	0.31	999	304
Cwal, Bwal	0.062	0.37	84	31
Cwal, Dsco	0.715	0.002	462	1
Cwal, Bsco	0.469	0.06	84	5
Cwal, Csco	0.639	0.002	462	1
Cwal, Asco	0.386	0.005	999	4
Dwal, Bwal	0.099	0.29	286	85
Dwal, Dsco	0.723	0.001	999	0
Dwal, Bsco	0.571	0.01	286	4
Dwal, Csco	0.767	0.001	999	0
Dwal, Asco	0.195	0.01	999	9
Bwal, Dsco	0.559	0.018	56	1
Bwal, Bsco	0.148	0.4	10	4
Bwal, Csco	0.352	0.04	84	3
Bwal, Asco	0.309	0.05	165	8
Dsco, Bsco	0.169	0.21	56	2
Dsco, Csco	-0.04	0.65	462	294
Dsco, Asco	0.533	0.003	999	2
Bsco, Csco	0.019	0.45	84	38
Bsco, Asco	0.269	0.09	165	15
Csco, Asco	0.467	0.001	999	0

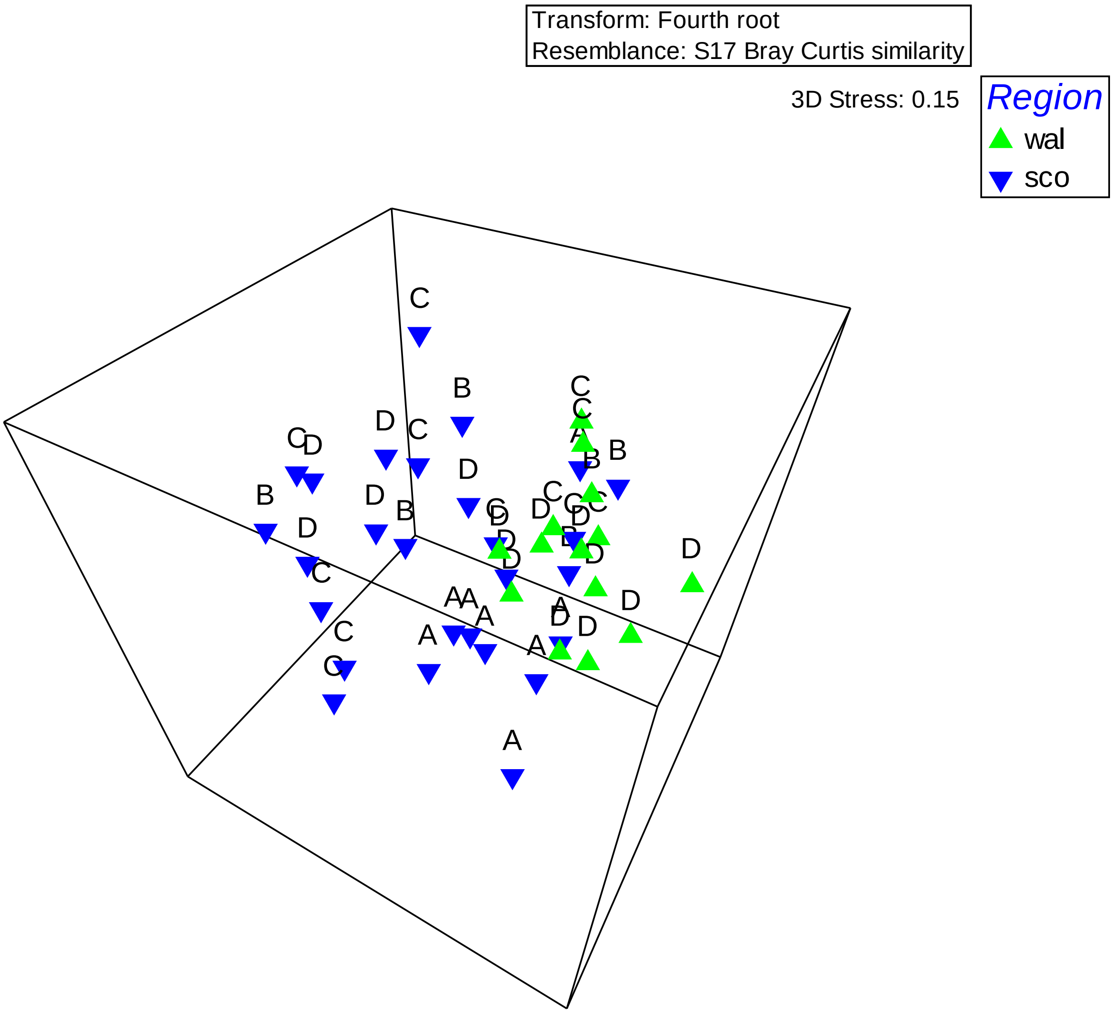
3.2 Testing for differences using multivariate ordination

3.2.1 Testing using non-metric Multidimensional Scaling (MDS)

To further investigate potential differences in reference communities an investigation was conducted using non-metric Multidimensional Scaling (MDS) (50 restarts, Kruskal fit) based on the Bray-Curtis matrix. MDS constructs a sample 'map' whose inter-point distances have the same rank-order (hence non-metric) as the corresponding dissimilarities between the samples (i.e. the Bray-Curtis matrix).

Results suggest MDS has high stress in 2-D space (0.21) and so the 3-D space ordination is shown (stress = 0.15) (Figure 6). Whilst the stress is still relatively high the ordination does clearly illustrate the separation between the Welsh and Scottish sites. Additionally, the clustering of Scottish sites in group A can be seen towards the near bottom corner of the ordination, supporting the results of the ANOSIM. Furthermore, the apparent random pattern of the other groups suggest a typology based on calcium is not required. Because the MDS stress is >0.1 more emphasis in interpretation should be placed on the CLUSTER analysis.

Figure 6: 3-D MDS plot of reference sites split according to region (Wales and Scotland) and Groups. Groups B, C and D are defined by the DAM typology for clear water types (<10 mg/l DOC). Group A is defined by humic sites (>10 mg/l DOC).

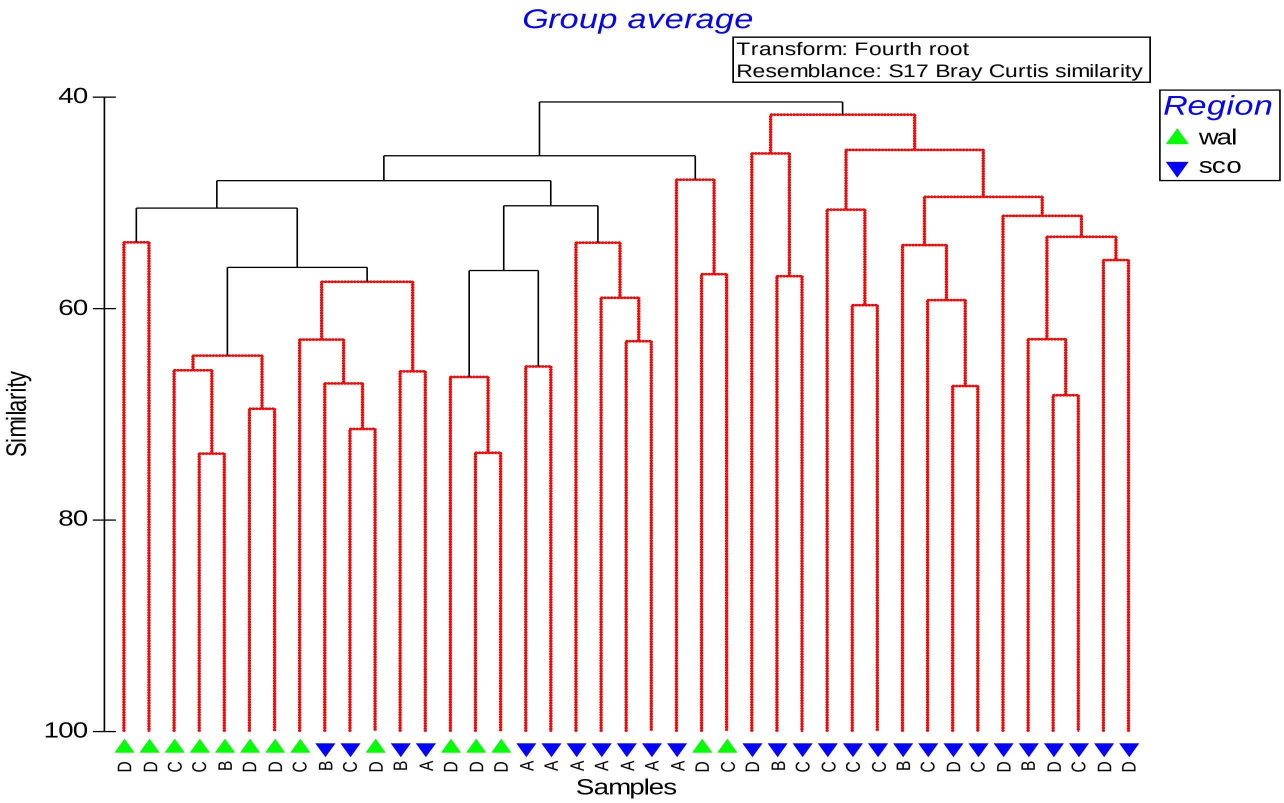


3.2.2 Testing using CLUSTER and SIMPROF analysis

CLUSTER analysis (group-average linkage), with SIMPROF (1000 permutations and *p*=0.05), to test for significance between the clusters, was used to compliment the MDS. Results of the CLUSTER analysis and SIMPROF groups are shown in Figure 7. Out of the 43 reference sites, 8 are in group A (DOC >10mg/l). Of these, 4 are in the same cluster, with no other sites, suggesting rivers of this type do have different reference communities. Two of the other sites with high DOC were placed together in a separate, although similar (>50% similarity), cluster. However, the differences between the two were significant according to SIMPROF. The most closely related cluster (although also significantly different) were three Welsh sites with lower DOC, pH >5.5 and high calcium. It is interesting to note that both these group A sites had lower DOC and higher pH (but lower ANC) than the other four sites. Despite the placing of these sites in a similar cluster, the results of the ANOSIM still consider these groups to be significantly different (Table 8, R = 0.195, p = 0.01) and the 3-D plot of the MDS shows the two group A sites to be closer to the other group A sites, than those in group D.

The CLUSTER analysis supports the MDS with a general separation of Scottish and Welsh sites. Although the split is clearly not precise, the right hand cluster consists of 100% Scottish sites. The cluster groups that split significantly from the right hand cluster, in the first fork of the dendrogram, are (excluding humic sites) 82% Welsh. This clearly suggests the need for a separate typology for Wales and Scotland. The fact the Scottish humic sites appear to be more closely related to Welsh clear-water sites, than to the Scottish clear-water sites (albeit not significantly) lends further support to the case of a separate humic typology in Scotland. The apparent random scatter of the DAM typological groups (i.e. groups B, C and D) across the clusters also suggests calcium does not drive differences in the community within these data.

Figure 7: Dendrogram of CLUSTER analysis with SIMPROF overlay derived from a Bray-Curtis similarity matrix of reference sites (significant divisions in the dendrogram are represented by black solid lines, where *p* =<0.05).



3.3 Final typology

Based on the findings of the ANOSIM, MDS and CLUSTER analysis the following typology is suggested;

Type 1 – Scottish sites >10 mg/l DOC

Type 2 – Scottish sites <10 mg/l DOC

Type 3 – Welsh sites

Due to the lack of English reference sites passing the screening process (mainly due to lack of species level data and ANC data) it is difficult to define a typology. A separate ANOSIM and CLUSTER analysis suggested the two English sites were more closely related to the Welsh sites. Based on this, it is suggested that the English typology and resulting boundaries use those derived for Wales. Clearly this is not an ideal situation and it is strongly recommended further data are gathered.

Scottish humic-water sites appear to have different reference communities. Lack of data made it impossible to test whether this was also the case for Wales (and England). More data need to be gathered for sites of this type.

4.0 Developing a type-specific reference community

A SIMPER test was used to investigate which individual species were driving the dissimilarity between types. The test ranks in order of importance each species by calculating their overall percentage contribution to the average dissimilarity *between* each type, enabling determination of which species are typical for each type. Furthermore, the test also lists which species contribute to the similarity *within* each type.

This list can then be used as the basis for developing a predicted reference community for each type.

4.1 Regional Type Community

The relative contribution of each species driving differences between the Scottish and Welsh reference communities can be seen in Table 9. Species typically found at Welsh references sites but not as commonly (or in such high abundances) at Scottish sites are predominantly stonefly; *Leuctra inermis, Isoperla grammatica, Chloroperla torrentium* and *Brachyptera risi.* Scottish reference sites (not including group A) typically have more mayfly; *Baetis rhodani, Alainites muticus, Rhithrogena semicolorata* and *Caenis rivulorum.* This might suggest, at reference sites, Scotland will have higher WFD-AWICsp scores than those in Wales.

Table 9: Contribution of species driving between-type ((Scotland (not inc. Group A) and Wales)) dissimilarity. SIMPER test, cutoff below 50%.

	Wales	Scotland				
Taxa	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Leuctra inermis	2.41	1.14	2.23	1.44	3.81	3.81
Isoperla grammatica	2.08	1.02	1.88	1.31	3.20	7.01
Simuliidae	1.34	1.53	1.76	1.15	3.00	10.00
Baetis rhodani	1.84	2.08	1.69	1.26	2.88	12.89
Chloroperla torrentium	1.95	0.77	1.68	1.51	2.87	15.75
Heptagenia lateralis	1.13	0.33	1.53	1.14	2.60	18.35
Brachyptera risi	0.90	0.48	1.49	1.05	2.54	20.89
Rhithrogena semicolorata	2.01	2.34	1.46	1.26	2.48	23.37
Hydropsyche siltalai	1.30	1.31	1.42	1.29	2.42	25.79
Caenis rivulorum	0.18	0.98	1.39	1.04	2.37	28.16
Alainites muticus	0.68	0.98	1.33	1.06	2.27	30.42
Elmis aenea	1.20	0.34	1.32	1.52	2.25	32.67
Ecdyonurus sp	1.07	0.70	1.32	1.26	2.24	34.91
Perla bipunctata	0.21	0.88	1.30	1.03	2.21	37.13
Oligochaeta	0.87	1.20	1.28	1.35	2.18	39.31
Chloroperla tripunctata	1.00	0.60	1.26	1.21	2.15	41.45
Hydroptila sp	0.00	0.91	1.26	1.29	2.15	43.60
Limnius volckmari	1.00	1.32	1.25	1.17	2.13	45.73
Chironomidae	2.21	1.88	1.22	1.34	2.09	47.82
Hydraena gracilis	0.90	0.13	1.18	1.04	2.02	49.84

4.1.1 Welsh Regional Type Community

The species that contribute to the similarity within the Welsh typology are listed in Table 10. Using a cut-off at 90%, the list represents a very typical faunal assemblage for Welsh reference sites, excluding rarer taxa, which may have more random effects upon metric scores. Sensitive species typical of Welsh reference sites driving the dissimilarities with both Scottish types are; *Ecdyonurus sp, Chloroperla tripunctata, Heptagenia lateralis* (Figure 8) and *Hydraena gracilis* (Figure 9).

The scoring taxa for WFD-AWICsp contained within Table 10 can now be used to construct an expected WFD-AWICsp metric value for Welsh sites that potentially are at risk from acidification (Table 11), resulting in a metric value of 7.65. This value can be used to form the basis of EQRs for Wales.

Table 10: Contribution of species accounting for similarity within the Welsh typology. SIMPER test, cutoff below 90%.

Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Isoperla grammatica	2.19	5.04	4.20	9.31	9.31
Leuctra inermis	2.40	4.50	2.02	8.32	17.63
Chironomidae	2.21	4.43	3.97	8.19	25.82
Amphinemura sulcicollis	1.97	4.00	4.06	7.39	33.21
Chloroperla torrentium	1.77	3.75	5.26	6.94	40.15
Rhithrogena semicolorata	1.93	3.08	1.43	5.69	45.84
Elmis aenea	1.41	3.01	4.73	5.56	51.40
Baetis rhodani	1.80	2.55	1.14	4.72	56.12
Simuliidae	1.28	2.02	1.15	3.74	59.86
Chloroperla tripunctata	1.19	1.84	1.15	3.40	63.26
Heptagenia lateralis	1.30	1.75	0.79	3.25	66.51
Limoniinae	1.02	1.66	1.18	3.07	69.58
Limnius volckmari	1.05	1.56	0.94	2.89	72.46
Rhyacophila dorsalis	1.08	1.43	0.96	2.65	75.12
Hydropsyche siltalai	1.11	1.41	0.77	2.61	77.73
Oligochaeta	0.85	1.27	0.95	2.35	80.08
Sericostoma personatum	0.78	1.04	0.77	1.92	81.99
Ecdyonurus sp	0.90	1.01	0.65	1.88	83.87
Brachyptera risi	0.89	0.96	0.61	1.78	85.65
Protonemura meyeri	0.77	0.95	0.80	1.76	87.41
Hydraena gracilis	0.89	0.91	0.63	1.68	89.09

Table 11: Expected reference WFD-AWICsp community and score for Welsh sites at risk from acidification

Predicted Taxa	**Predicted Log Abundance**	**Score**
Isoperla grammatica	B	6
Amphinemura sulcicollis	B	4
Leuctra inermis	B	4
Chloroperla torrentium	A	5
Rhithrogena semicolorata	B	11
Baetis rhodani	B	11
Elmis aenea	A	8
Hydropsyche siltalai	A	8
Sericostoma personatum	A	10
Ecdyonurus sp	A	10
Limnius volckmari	A	8
Rhyacophila dorsalis	A	7
Heptagenia lateralis	A	8
Chloroperla tripunctata	A	8
Protonemura meyeri	A	5
Brachyptera risi	A	7
Hydraena gracilis	A	10
∑Predicted taxa scores		130

∑Predicted taxa scores/N	**7.65**

Figure 8: MDS of reference sites split according to types with bubble-plot of presence and abundance of *Heptagenia lateralis* overlaid.

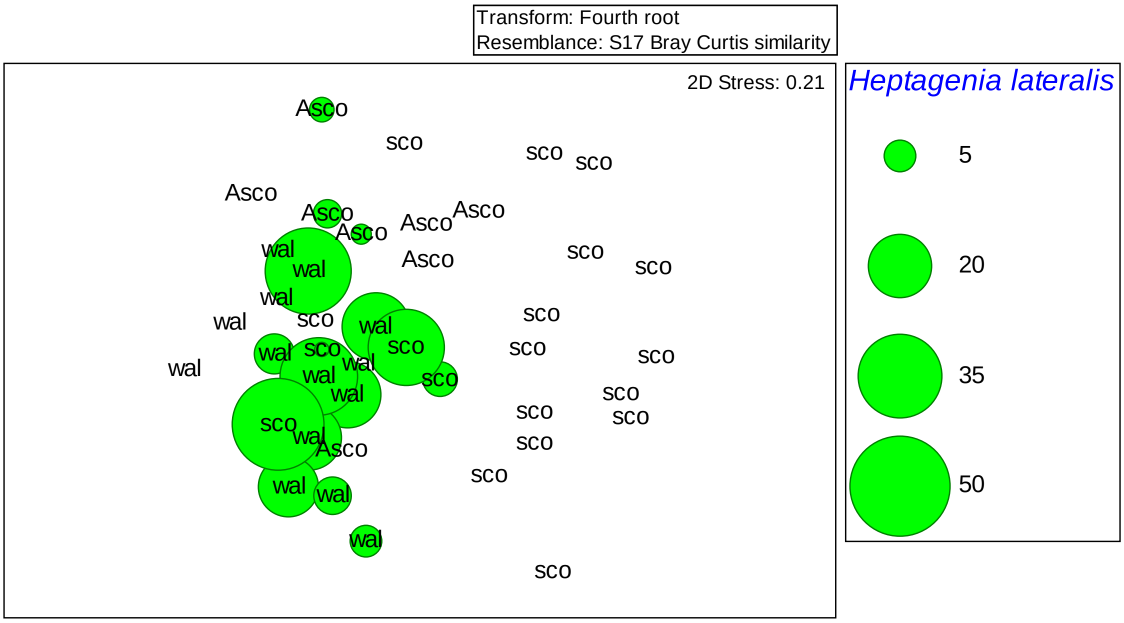
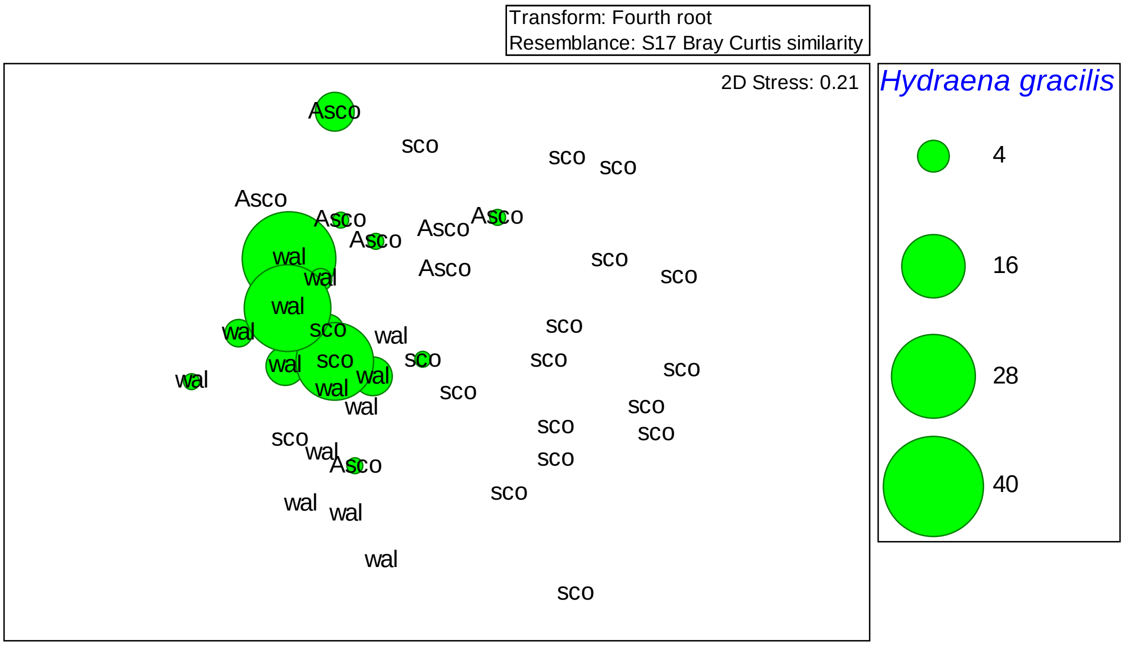


Figure 9: MDS of reference sites split according to types with bubble-plot of presence and abundance of *Hydraena gracilis* overlaid.



4.1.2 Scottish Regional Type Community

The species that contribute to the similarity within the Scottish typology are listed in Table 12. This does not include Group A sites i.e. Sites with DOC >10 mg/l. Using a cut-off at 90%, the list represents a very typical faunal assemblage for Scottish reference sites, again excluding rarer taxa, which may have more random effects upon metric scores. Sensitive species typical of Scottish reference sites driving the dissimilarities with both the other types are; *Baetis rhodani, Rhithrogena semicolorata, Caenis rivulorum* (Figure 10), *Alainites muticus* and *Perla bipunctata* (Figure 11).

The scoring taxa for WFD-AWICsp contained within Table 12 can now be used to construct an expected WFD-AWICsp metric value for Scottish sites that potentially are at risk from acidification (Table 13) resulting in a metric value of 8.61. This value can be used to form the basis of EQRs for Scotland.

Table 12: Contribution of species accounting for similarity within the Scottish typology (not inc Group A). SIMPER test, cutoff below 90%.

Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Rhithrogena semicolorata	2.32	5.63	2.55	12.45	12.45
Chironomidae	1.96	4.43	3.45	9.79	22.24
Amphinemura sulcicollis	1.93	4.38	2.37	9.69	31.94
Baetis rhodani	2.05	4.33	1.50	9.58	41.52
Hydropsyche siltalai	1.43	2.62	1.27	5.79	47.31
Simuliidae	1.52	2.07	0.89	4.57	51.89
Leuctra inermis	1.45	2.05	0.92	4.54	56.43
Limnius volckmari	1.21	2.04	1.07	4.51	60.94
Chloroperla torrentium	1.18	1.91	0.93	4.22	65.15
Oligochaeta	1.14	1.88	0.83	4.16	69.32
Isoperla grammatica	1.20	1.59	0.74	3.51	72.83
Rhyacophila dorsalis	0.82	1.37	0.84	3.03	75.85
Ecdyonurus sp	0.90	1.31	0.64	2.90	78.75
Baetis muticus	0.97	1.22	0.65	2.71	81.46
Caenis rivulorum	0.91	1.09	0.57	2.42	83.88
Perla bipunctata	0.76	0.94	0.50	2.09	85.97
Hydroptila sp	0.70	0.88	0.58	1.94	87.91
Dinocras cephalotes	0.60	0.60	0.44	1.33	89.23

Table 13: Expected reference WFD-AWICsp community and score for Scottish sites (not including Group A) at risk from acidification

Predicted Taxa	**Predicted Log Abundance**	**Score**
Rhithrogena semicolorata	B	11
Amphinemura sulcicollis	B	4
Baetis rhodani	B	11
Hydropsyche siltalai	A	8
Leuctra inermis	A	5
Limnius volckmari	A	8
Chloroperla torrentium	A	5
Isoperla grammatica	A	7
Rhyacophila dorsalis	A	7
Ecdyonurus sp	A	10
Alainites muticus	A	12
Caenis rivulorum	A	12
Perla bipunctata	A	12
∑Predicted taxa scores		112
∑Predicted taxa scores/N		**8.61**

Figure 10: MDS of reference sites split according to types with bubble-plot of presence and abundance of *Caenis rivulorum* overlaid.

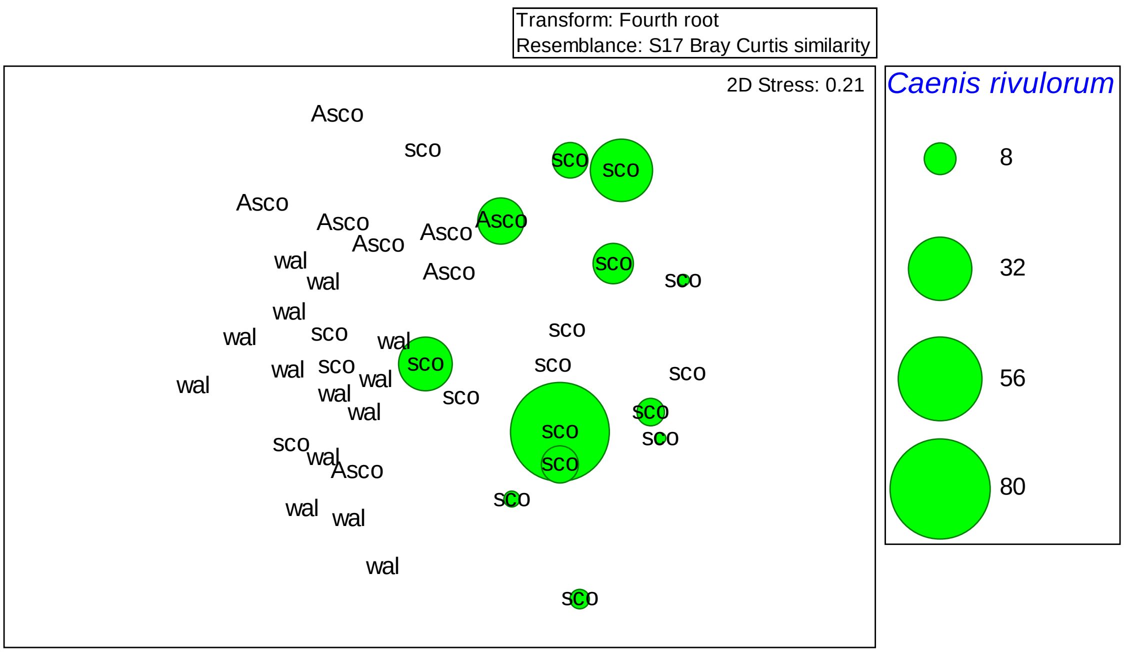
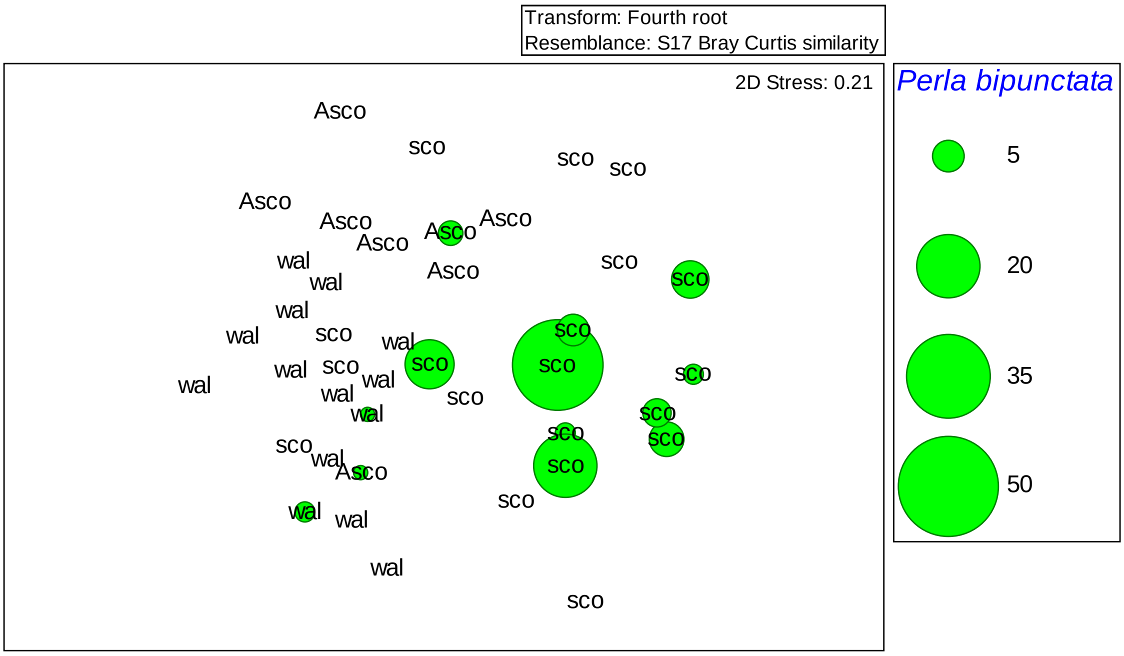


Figure 11: MDS of reference sites split according to types with bubble-plot of presence and abundance of *Perla bipunctata* overlaid.



4.1.3 Scottish Humic Type Community

The relative contribution of each species driving differences between the Scottish humic reference type and the Scottish and Welsh reference communities can be seen in Tables 14 and 15 respectively. Species typically found at humic Scottish references sites but not as commonly (or in such high abundances) at other Scottish sites are riffle beetles (Elmidae); *Elmis aenea* (Figure 13), *Oulimnius sp* and, to a lesser extent, *Limnius volckmari* (Figure 12). The same can be true when compared to Welsh sites, although *L.volckmari* contributes more to the differences than either *E.aenea* or *Oulimnius*. This suggests the presence of high abundances of Elmidae are typical of naturally humic waters with low pH levels, but high ANC. Given these species are considered generally sensitive, they are likely to be good indicators of anthropogenic acidification at acid water sites. Other species typical of these sites are the caddisfly, *Lepidostoma hirtum* and *Hydropsyche siltalai*, moderately sensitive taxa that can occur at low pH where ANC is high. Scottish reference sites at lower DOC levels typically have more mayflies e.g. *Rhithrogena semicolorata*, *Baetis rhodani* and *Caenis rivulorum.* In the case of the former two, this also applies to the Welsh sites, despite them being less common than in Scotland. This suggests that mayflies generally are sensitive not only to low ANC but pH too, where abundance and diversity are lower at naturally acid sites, as well as acidified waterbodies. An exception to this appears to be *Heptagenia sulphurea*, which occurs more frequently at the Scottish humic sites. Again, this may suggest this species is a good indicator of acidification in highly humic, low pH, conditions.

The scoring taxa for WFD-AWICsp contained within Table 16 can now be used to construct an expected WFD-AWICsp metric value for Scottish humic sites that potentially are at risk from acidification (Table 17) resulting in a metric value of 7.38. This value can be used to form the basis of EQRs for Scotland. This expected value is noticeably lower than sites in Scotland below 10 mg/l DOC (8.61) reflecting the lower number of sensitive taxa that can tolerate the low pH in humic-waters at reference condition.

Table 14: Contribution of species driving between-type (Scottish humic sites and other Scottish sites) dissimilarity. SIMPER test, cutoff below 50%.

Taxa	Scot Clear Av.Abund	Scot Humic Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Elmis aenea	0.34	1.86	1.95	1.73	3.30	3.30
Oulimnius	0.26	1.73	1.87	2.17	3.18	6.49
Rhithrogena semicolorata	2.34	1.01	1.80	1.33	3.06	9.55
Simuliidae	1.53	1.80	1.79	1.18	3.04	12.60
Baetis rhodani	2.08	1.45	1.66	1.24	2.82	15.41
Leuctra inermis	1.14	1.10	1.57	1.27	2.66	18.08
Chloroperla torrentium	0.77	2.02	1.56	1.57	2.65	20.73
Limnius volckmari	1.32	2.39	1.55	1.30	2.63	23.36
Lepidostoma hirtum	0.69	1.75	1.52	1.52	2.59	25.95
Isoperla grammatica	1.02	1.93	1.48	1.25	2.51	28.45
Hydropsyche siltalai	1.31	2.14	1.40	1.50	2.37	30.82
Heptagenia sulphurea	0.25	1.30	1.39	1.17	2.36	33.18
Caenis rivulorum	0.98	0.34	1.26	1.04	2.15	35.33
Protonemura meyeri	0.23	1.16	1.26	1.67	2.15	37.48
Empididae	0.43	1.22	1.17	1.47	1.99	39.47
Hydroptila sp	0.91	0.34	1.17	1.32	1.99	41.46
Chironomidae	1.88	2.51	1.16	1.38	1.97	43.43
Baetis muticus	0.98	0.50	1.16	1.02	1.97	45.40
Brachyptera risi	0.48	0.78	1.16	0.90	1.96	47.37
Oligochaeta	1.20	1.87	1.14	1.25	1.94	49.31

Table 15: Contribution of species driving between-type (Scottish humic sites and Welsh sites) dissimilarity. SIMPER test, cutoff below 50%.

Taxa	Wales Av.Abund	Scot Humic Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Leuctra inermis	2.41	1.10	2.02	1.61	3.75	3.75
Lepidostoma hirtum	0.30	1.75	1.67	1.65	3.10	6.85
Limnius volckmari	1.00	2.39	1.65	1.48	3.06	9.92
Baetis rhodani	1.84	1.45	1.57	1.21	2.91	12.82
Rhithrogena semicolorata	2.01	1.01	1.48	1.38	2.74	15.56
Oulimnius	0.55	1.73	1.41	1.67	2.61	18.18
Empididae	0.00	1.22	1.38	1.93	2.56	20.74
Simuliidae	1.34	1.80	1.37	1.17	2.55	23.29
Heptagenia sulphurea	0.00	1.30	1.32	1.13	2.45	25.73
Elmis aenea	1.20	1.86	1.24	1.58	2.31	28.04
Heptagenia lateralis	1.13	0.46	1.21	1.15	2.25	30.29
Oligochaeta	0.87	1.87	1.18	1.47	2.19	32.48
Chloroperla tripunctata	1.00	0.00	1.11	1.16	2.06	34.54
Hydropsyche siltalai	1.30	2.14	1.11	1.10	2.05	36.59
Ecdyonurus sp	1.07	0.76	1.10	1.14	2.03	38.62
Brachyptera risi	0.90	0.78	1.08	1.14	2.00	40.61

Hydraena gracilis	0.90	0.59	0.97	1.11	1.79	42.41
Sericostoma personatum	0.57	0.86	0.97	1.20	1.79	44.20
Chironomidae	2.21	2.51	0.96	1.23	1.79	45.98
Hydropsyche pellucidula	0.00	0.92	0.95	1.38	1.77	47.75
Plectrocnemia conspersa	0.81	0.50	0.92	1.18	1.70	49.45

Table 16: Contribution of taxa accounting for similarity within the humic Scottish typology (>10 mg/l DOC). SIMPER test, cutoff below 90%.

Taxa	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Chironomidae	2.27	3.85	4.22	7.93	7.93
Limnius volckmari	2.31	3.84	3.16	7.91	15.84
Isoperla grammatica	1.88	3.80	4.51	7.83	23.67
Amphinemura sulicollis	2.14	3.64	3.29	7.50	31.17
Hydropsyche siltalai	1.78	2.89	1.53	5.95	37.12
Oligochaeta	1.66	2.53	1.63	5.21	42.33
Simuliidae	2.03	2.43	1.23	5.01	47.34
Chloroperla torrentium	1.64	2.37	1.49	4.89	52.23
Elmis aenea	1.64	2.29	1.53	4.73	56.95
Oulimnius	1.46	2.09	1.57	4.32	61.27
Baetis rhodani	1.60	1.97	0.87	4.05	65.32
Rhyacophila dorsalis	1.22	1.95	1.61	4.02	69.35
Rhithrogena semicolorata	1.58	1.90	0.90	3.93	73.28
Protonemura meyeri	1.06	1.49	1.02	3.07	76.35
Leuctra inermis	1.32	1.37	0.72	2.82	79.17
Empididae	1.04	1.07	0.70	2.70	81.87
Lepidostoma hirtum	1.31	0.81	0.72	2.21	84.08
Heptagenia sulphurea	1.13	0.73	0.70	1.66	85.74
Hydraena gracilis	1.70	0.60	0.72	1.50	87.24
Athripsodes	0.60	0.60	0.50	1.23	88.47
Brachyptera risi	1.09	0.58	0.47	1.20	89.68

Table 17: Expected reference WFD-AWICsp community and score for Scottish humic-water sites (>10 mg/l DOC) at risk from acidification

Predicted Taxa	**Predicted Log Abundance**	**Score**
Limnius volckmari	B	9
Isoperla grammatica	B	6
Amphinemura sulcicollis	B	4
Hydropsyche siltalai	A	9
Chloroperla torrentium	A	5
Elmis aenea	A	8
Oulimnius	A	5
Baetis rhodani	A	10
Rhyacophila dorsalis	A	7
Rhithrogena semicolorata	A	10
Protonemura meyeri	A	5
Leuctra inermis	A	5
Lepidostoma hirtum	A	8
Heptagenia sulphurea	A	10

Hydraena gracilis	A	10
Brachyptera risi	A	7
∑Predicted taxa scores		118
∑Predicted taxa scores/N		**7.38**

Figure 12: MDS of reference sites split according to types with bubble-plot of presence and abundance of *Limnius volckmari* overlaid.

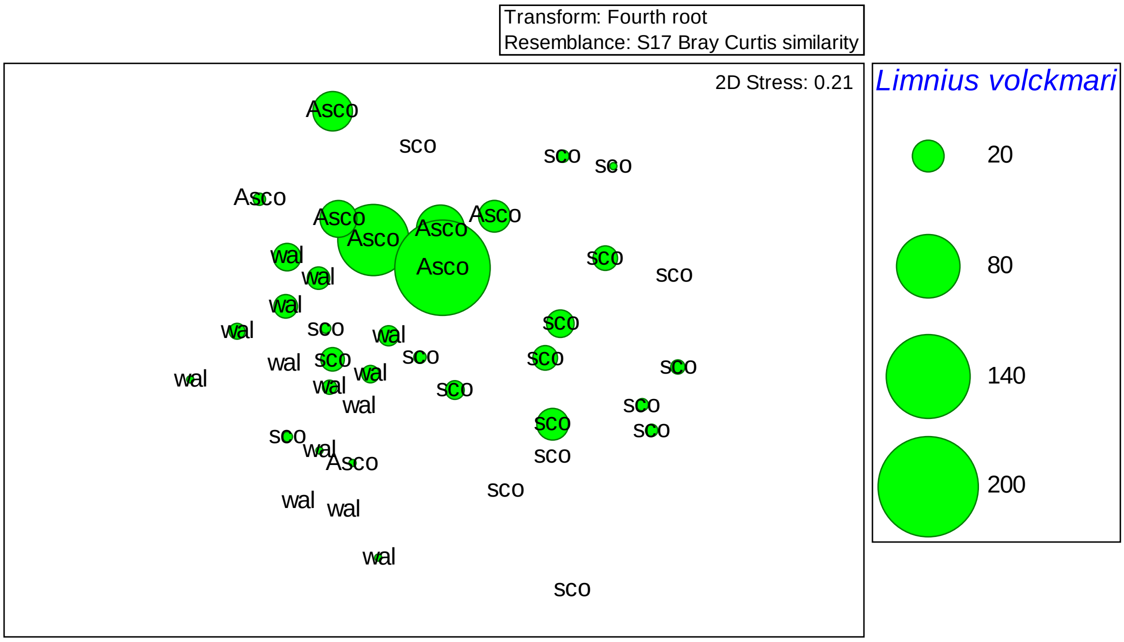
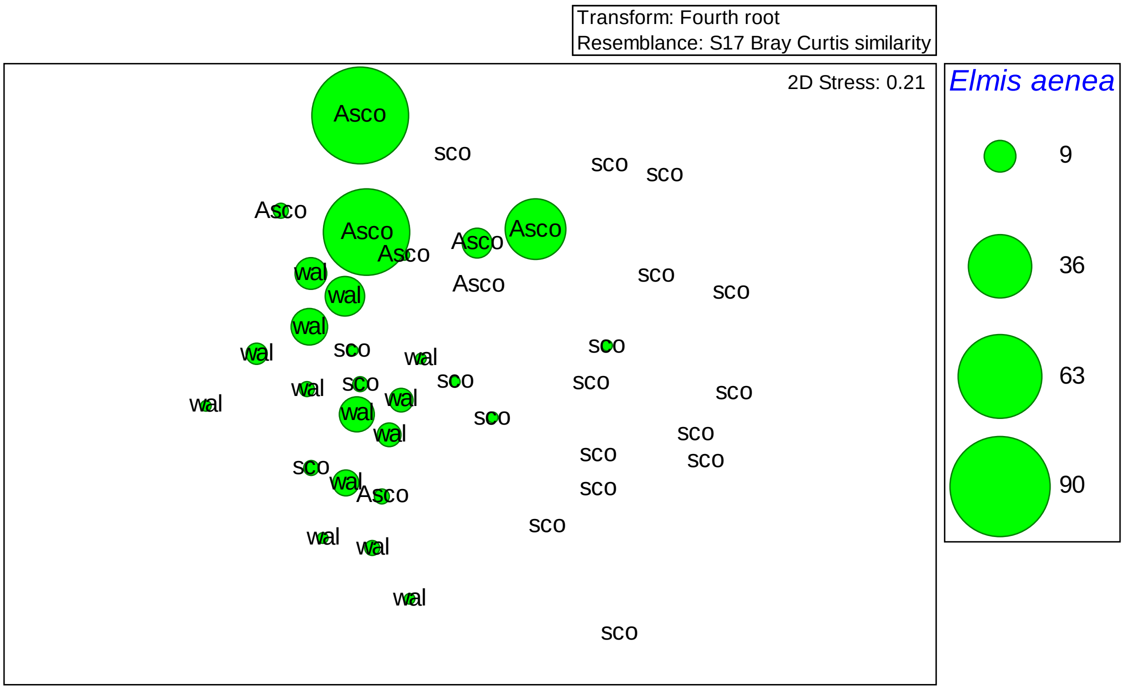


Figure 13: MDS of reference sites split according to types with bubble-plot of presence and abundance of *Elmis aenea* overlaid.



5.0 Boundary Development for Scottish Humic Sites

For a site to be included in the boundary development it had to have a minimum of four chemical samples taken in the previous two years from the date of the biological sample. Thirty three sites met these criteria and had DOC >10 mg/l.

To investigate potential step-changes in the pressure gradient the response of the community was analysed using Lowess Smoothing (degrees of smoothing = 0.5, steps = 2) in Minitab 15. In addition to WFD-AWICsp, this included diversity (number of individuals, number of taxa, Shannon diversity and number of functional groups) and function, where percentage of taxa possessing specific feeding traits were used as a proxy for functional processes. Taxa were assigned to a functional group following AQEM (2004) and three functional groups were used; grazers, shredders and parasites. Previous research suggests that grazers are sensitive to acidification (e.g. Ledger & Hildrew, 2005). Conversely, acidified sites are expected to have higher numbers of shredders (Petrin *et al.,* 2007) and evidence from lakes stressed from acidification suggest there may be higher numbers of parasites (McFarland *et al,* 2009).

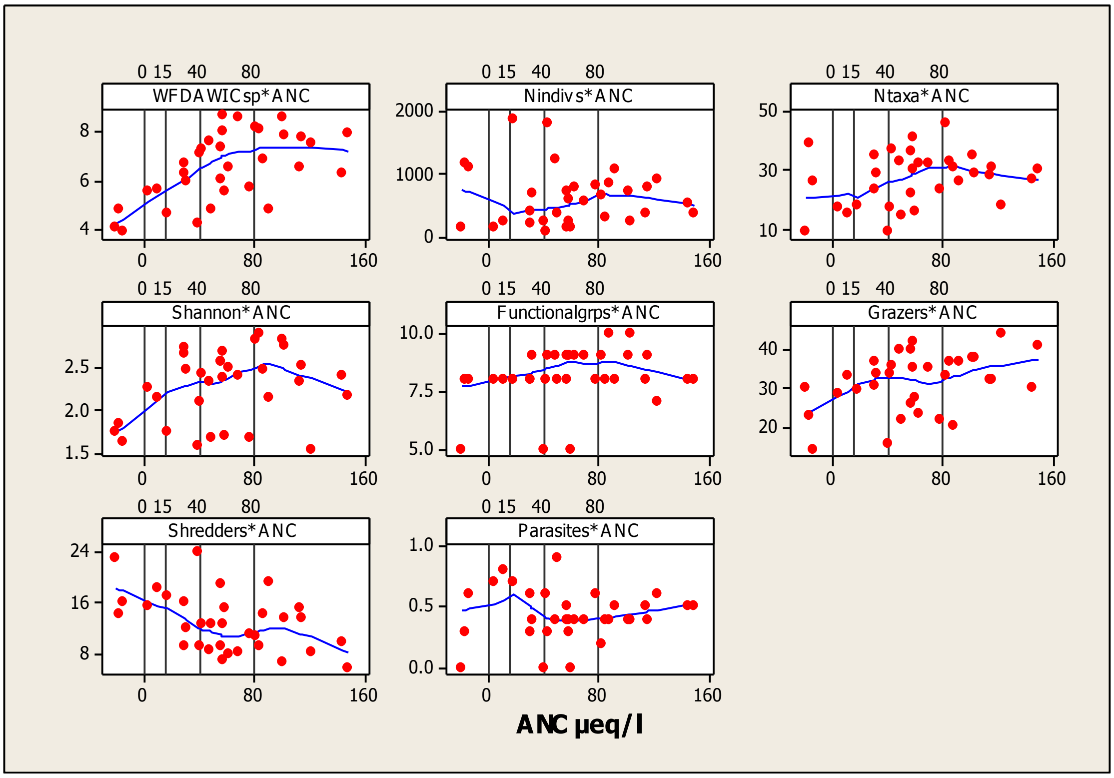
5.1 High-Good Boundary

The High-Good boundary was chosen at ANC 80 µeq/l. At first this may appear high as there are few empirical datasets to suggest that sites with ANC at this level will be anything but high status. Indeed, the UCL damage matrix (Monteith and Simpson, 2007) classifies all sites with ANC 80 µeq/l as high status (irrespective of Ca levels), as does the LAMM method for lakes (McFarland *et al*, 2009). Despite this, there is evidence from the community that changes begin around this level. Rivers at risk from acidification are known for their episodic acid events. The inclusion of sites in this analysis depends on having a minimum of 4 chemical samples from the preceding two years. This was necessary to ensure the inclusion of enough sites for realistic boundary setting. However, it is still possible episodic events were missed. With more data from the same window, these events are more likely to be picked up, which would drive down ANC values. In turn, this is likely to lower threshold responses along the x-axis. Therefore, in reality, the ANC figure for community change from good to high status is likely to be much lower.

Above the 80 µeq/l threshold WFD-AWICsp remains at a relatively constant level (approximately 7.1-7.4), similar to the reference value (7.38) (Figure 14). However, at ANC 80 µeq/l many of the smoothing lines begin to drop and, for some metrics, is the start of a consistent drop to the most acidified sites. Below this threshold we begin to see the first noticeable, consistent changes in WFD-AWICsp. This is supported by consistent changes in diversity. All the diversity indicators are non-linear in response to pressure, with broadly unimodal responses peaking around 90-100 µeq/l . We can hypothesise over the causes of this. It may be that these rivers have naturally slightly lowered ANC, enabling taxa associated with more acid, and perhaps oligotrophic conditions, to colonise and compete effectively with taxa associated with circumneutral waters. At approximately ANC 80 µeq/l, Shannon diversity, the number of individuals and the number of functional groups begin to fall. Shannon diversity begins to drop, the start of a consistent decline to the most acidified sites, mirroring WFD-AWICsp. The number of taxa remains the same, until 70µeq/l , before falling. The most sensitive taxa remain until this point but often with lower abundances. This reflects the small decline in WFD-AWICsp and resulting changes in community evenness, affecting Shannon diversity.

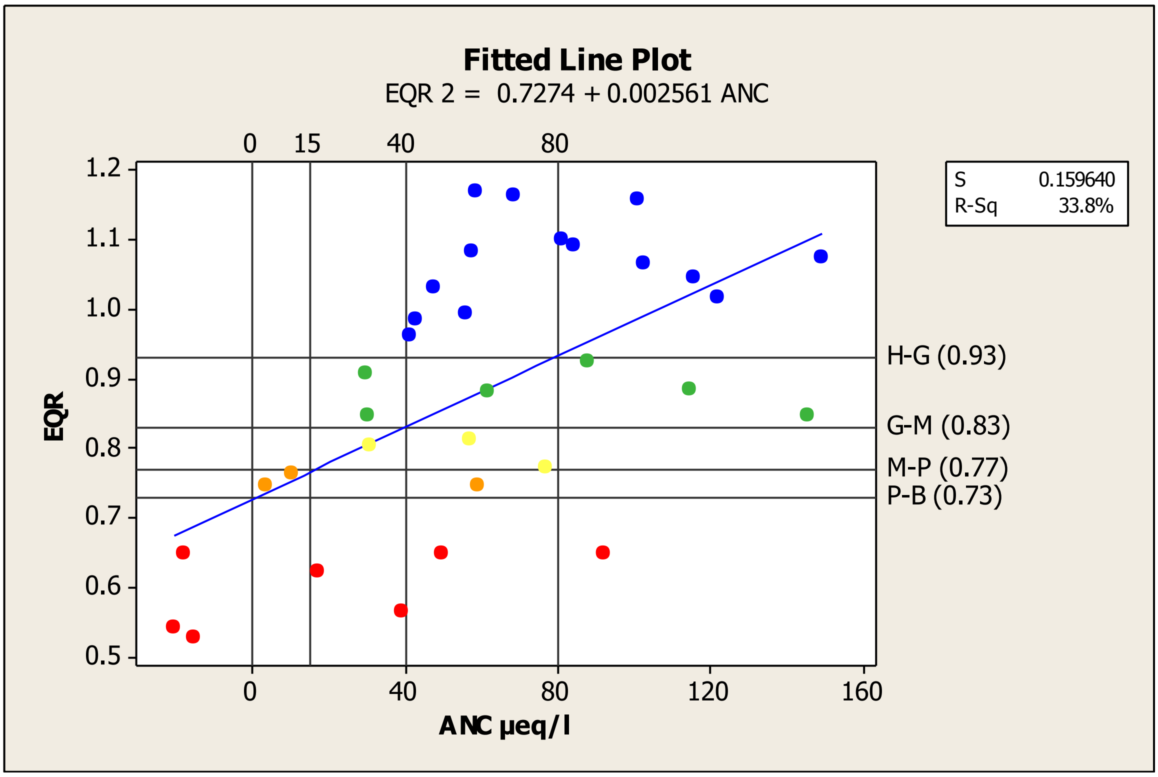
Grazers show a more linear response from ANC 150 to 80 µeq/l. This is broadly mirrored in the opposite direction by shredders. The declining grazer population coupled with increases in shredders as ANC declines may reflect the shift from autochthonous to allochthonous carbon suggesting changes in ecosystem processes, with reduced primary production and external carbon sources becoming increasingly important. However, at this point along the pressure gradient, this may represent a natural community response to decreases in nutrients within the system, rather than a response to stress from acidification. The parasitic contribution to the community declines marginally from ANC 150 to 80 µeq/l, in contrast to the expected response, perhaps supporting the case for low level of stress along this part of the pressure gradient. Generally increases in parasites within a community only becomes significant towards more stressed environments (Lafferty & Kuris,1999) and so at the high-good boundary we would not expect increases in this functional group.

Figure 14: Scatterplots of WFD-AWICsp, diversity metrics and selected functional groups verses ANC, at Scottish humic sites (>10 mg/l DOC), with Lowess fit. Breakpoints are represented by reference lines along the x-axis.



The threshold figure of 80 µeq/l can be applied to a linear regression equation between WFD-AWICsp EQRs and ANC (Figure 15). Using the x-axis as the predictor results in a High-Good boundary of 0.93. The regression fit is relatively poor (R^2 = 0.34, P = <0.001) and so this boundary was compared to the boundary derived from the damage matrix using the same method developed for lakes using LAMM (McFarland *et al*, 2009). This method results in a very similar boundary (0.95) increasing confidence that 0.93 is ecologically relevant.

Figure 15: Linear regression of WFD-AWICsp EQRs verses ANC at Scottish humic sites. References lines are derived from Figure 14 and are used to predict EQR boundaries on the y-axis (● = High, ● = Good, ● = Moderate, ● = Poor, ● = Bad).



5.2 Good-Moderate Boundary

The Good-Moderate boundary was chosen at ANC 40 µeq/l. Again this may appear rather high although the UCL damage matrix (Monteith and Simpson, 2007) classifies sites at ANC 40 µeq/l as anything from moderate to high status depending on calcium levels. Again episodic acid events missed in sampling may mean that actual ANC values that are driving community change are considerably less than this.

At the 40 µeq/l threshold the smoothing line is some way down a continuous linear decline to the most acidified sites. Diversity also appears to be declining for NTaxa, functional groups and Shannon Diversity. Immediately below 40 µeq/l the angle of the line for NTaxa increases, suggesting increasing pressure. The line for grazers, having levelled off in good status, falls rapidly below 40 µeq/l. Conversely the percentage of shredders increases. This suggests a functional shift to allochthonous carbon sources typical of acidified systems. Parasites show a marked increase below 40 µeq/l and this may indicate the start of significant community stress.

The boundary was again compared to the boundary derived from the LAMM boundary setting method. This method results in a very similar boundary (0.85) increasing confidence that 0.83 is ecologically relevant.

5.3 Moderate-Poor Boundary

The Moderate-Poor boundary was set using ANC 15 µeq/l. At this level WFD-AWICsp continues to decline although the UCL damage matrix generally classifies sites as moderate at this level of ANC. However, as discussed previously, the actual levels of ANC during periods of acid stress are likely to be lower for sites near this threshold. Below 15 µeq/l the number of individuals begins to rise and this may support the hypothesis that as pressure increases, the fewer taxa that remain can proliferate with reduced resource competition. However, this response model is based largely on organic pollution and the line is influenced strongly by only two sites, although each is dominated by very high abundances of the tolerant taxon, *Amphinemura sulcicollis* (Benmeal = 659 individuals, Burn 11 = 645 individuals). At this boundary the decline in the number of taxa levels off. The loss of highly sensitive and sensitive taxa occurs in the moderate band. Below 15 µeq/l only a few moderately senstive taxa, at low abundances, remain. Shannon diversity, however, continues to fall. Given the loss of highly sensitive and sensitive taxa in moderate status, this drop reflects changes in eveness, rather than taxonomic composition. Below this threshold the tolerant taxa become more dominant. The diversity of functional groups also decreases, although not as steeply as the decline through the moderate band. Grazers, largely characterised by sensitive taxa, continue to decline. The parasitic loading peaks near this threshold, suggesting high levels of community stress.

A comparison with the LAMM boundary setting method results in exactly the same EQR (0.77).

5.4 Poor-Bad Boundary

The Poor-Bad boundary has been set at ANC 0 µeq/l resulting in an EQR of 0.73. There are few data to determine the location of the boundary here and so caution should be applied to this. Using a threshold of 0 µeq/l, a figure that suggests the lack of any buffering, seems logical. Additionally the UCL damage matrix classifies sites at this level of ANC as bad, excepting sites with very low levels of calcium (<20 ueq/l). The use of this threshold is supported by the very low WFD-AWICsp for the three sites below the threshold. Two of these sites have no moderately sensitive taxa (one has a single specimen of *Limnius volckmari*) and all have only one moderately tolerant taxon. The rest of the community consists of tolerant and highly tolerant taxa only, often in high abundances. There is also a noticeable decline in Shannon diversity; all three sites having very low scores. Grazers continue to fall with a corresponding increase in shredders.

Comparisons with the LAMM boundary setting method shows moderate differences between the EQRs (0.73 compared to 0.67 for the LAMM method). Due to the lack of data and the differences with the LAMM method, care must be taken with this boundary.

5.5 Boundary Evaluation

5.5.1 Contour Plots

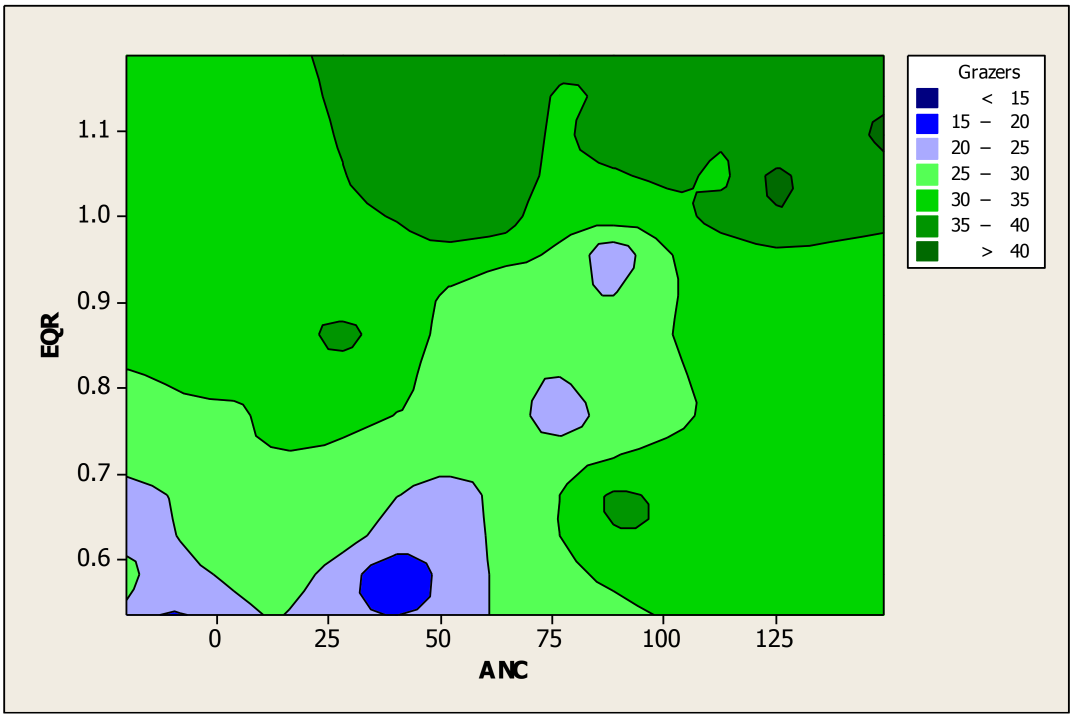
For a further evaluation of the location of the boundaries, contour plots have been used (distance method, power = 2) where the Z variables are for significant metrics (i.e. those used in figure 14) v ANC, using linear regression, after testing for normality using Ryan-Joiner and Bonferroni correction (significance where p = <0.006).

Shredders was the only metric (excepting WFD-AWICsp) found to respond significantly to ANC. However, the relationship between grazers and ANC was significant at $p = 0.006$ and as Bonferroni correction sets a notoriously 'high bar' this group is also discussed. Figure 16 indicates that high proportions of grazers (>35%) generally only occur above EQR 0.95, although as these high abundances can be found at comparatively low ANC levels, it does suggest a high-good boundary of 0.94 reflects subtle changes in grazer numbers. Below ANC 80 µeq/l and EQR 0.94, the community has lower levels (sites with 25-35% increasingly common). This change is broadly mirrored by the rise in shredders (Figure 17). Below ANC 40 µeq/l and EQR 0.84 (i.e. below the good-moderate boundary), sites will invariably have shredders >12%, increasingly well over 16%, and generally have grazers with <30%. Typical expected grazer and shredder contributions for each status class can be seen in Table 18.

Table 18: Typical percentage grazer and shredder contributions to classes for Scottish humic sites

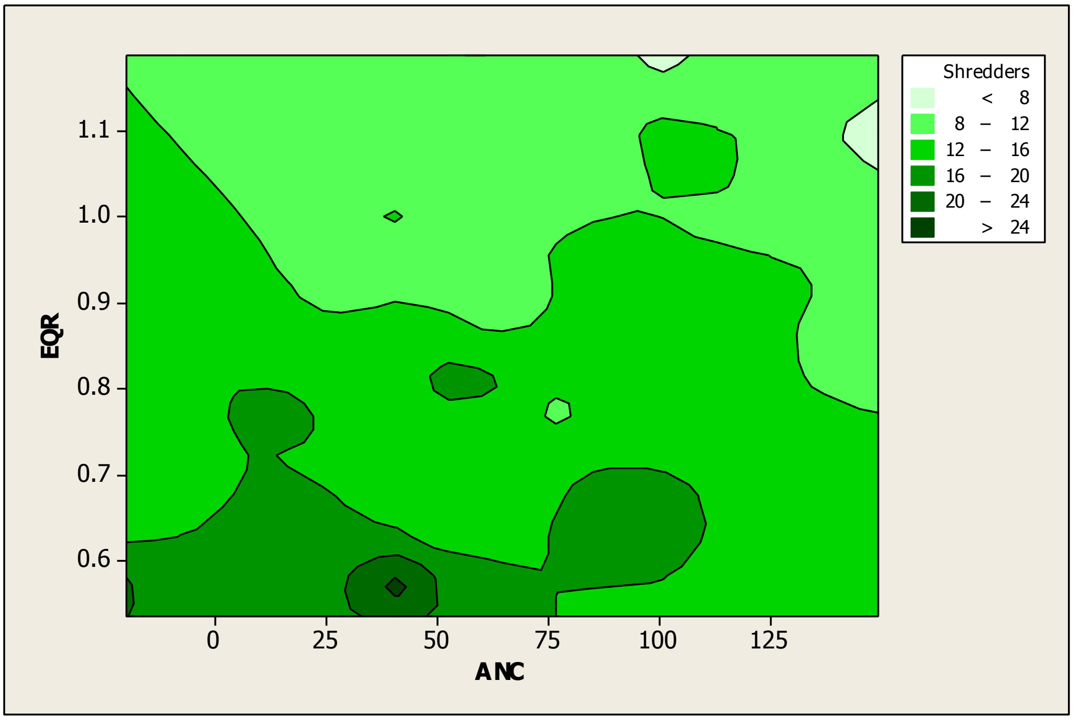
Status Class	Grazers (Typical % Contribution)	Shredders (Typical % Contribution)
High	>35%	<12%
Good	25-35%	12-16%
Moderate	25-35%	>16%
Poor-Bad	20-25%	16-20%

Figure 16: Contour plot of EQR verses ANC with percentage grazers overlaid as the Z-variable



Grazers
< 15
15 – 20
20 – 25
25 – 30
30 – 35
35 – 40
> 40
EQR
1.1
1.0
0.9
0.8
0.7
0.6
0
25
50
75
100
125
ANC

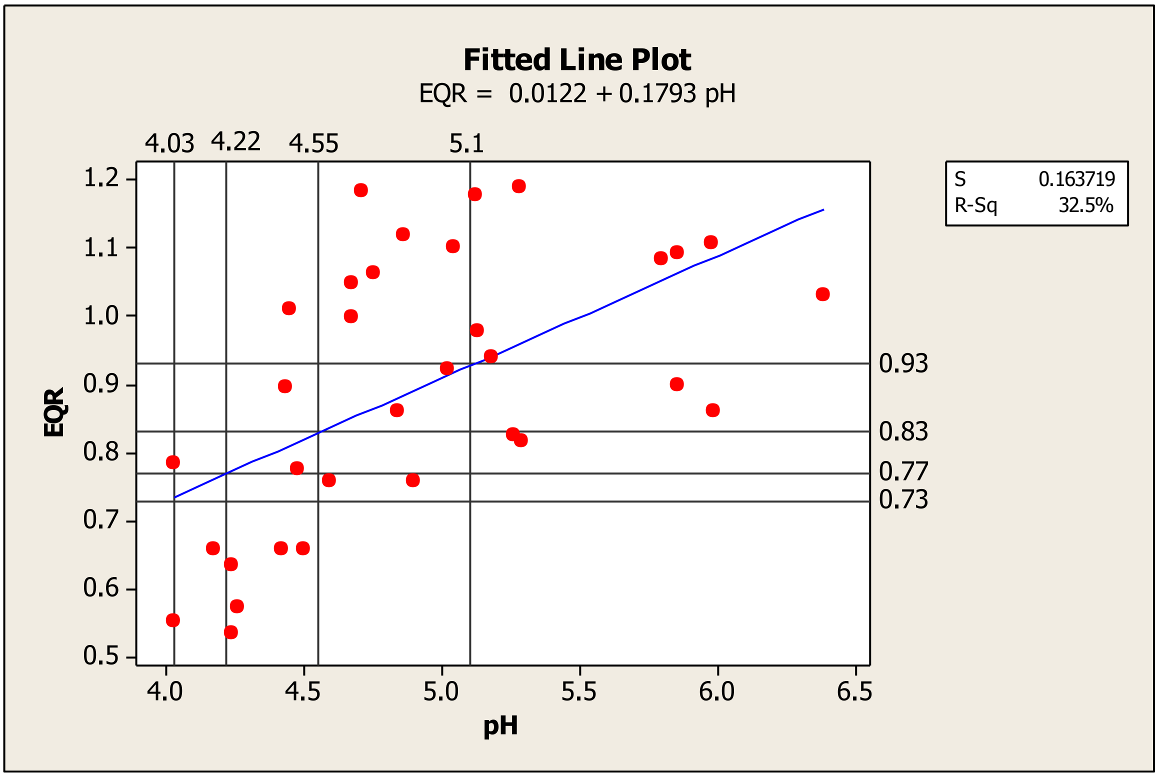
Figure 17: Contour plot of EQR verses ANC with percentage shredders overlaid as the Z-variable



5.5.2 Predicted pH boundaries

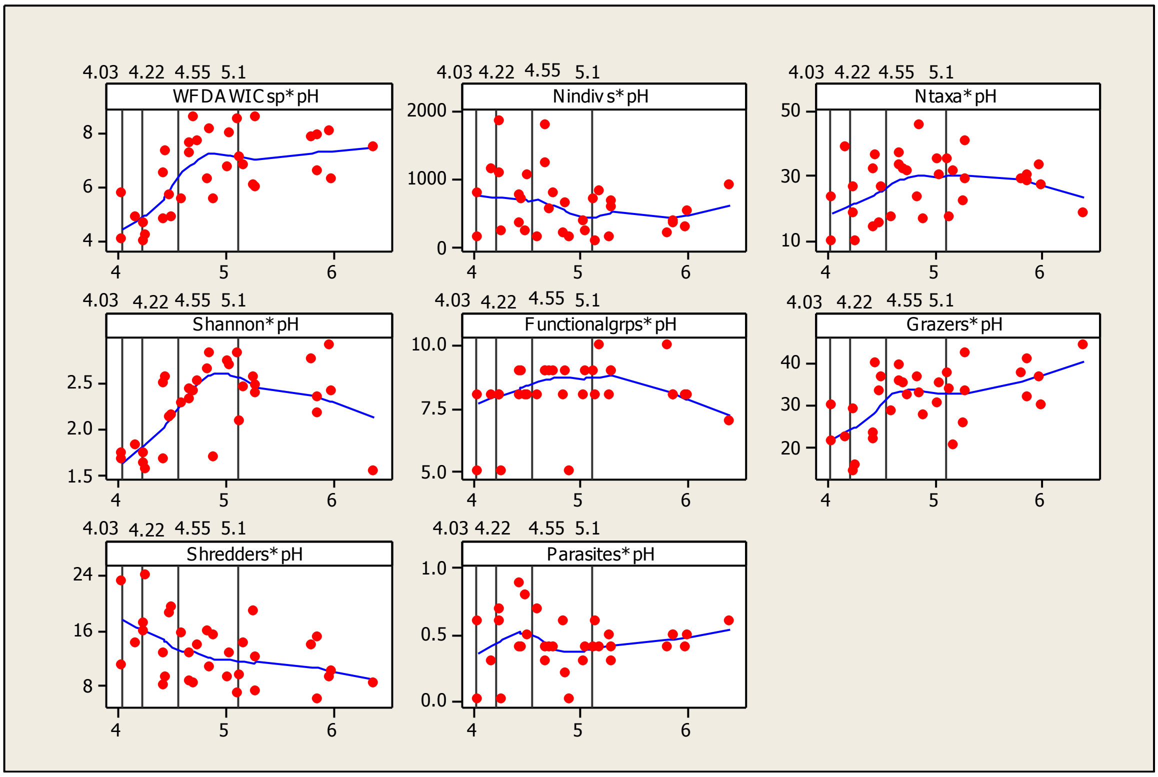
As a further evaluation of the validity of the boundaries, the EQR values have been used to predict pH values from a linear regression (Figure 18). A certain amount of caution is required as, although the relationship is highly significant, it is rather weak ($R^2 = 0.33$, $P = <0.01$). The resulting boundaries result in pH values as 5.1 (High-Good), 4.55 (Good-Moderate), 4.22 (Moderate-Poor) and 4.03 (Poor-Bad). These values can then be compared to the same set of metrics used to develop boundaries using ANC and step-changes can again be investigated using Lowess smoothing (degrees of smoothing = 0.5, steps = 2).

Figure 18: Linear regression of WFD-AWICsp verses pH at Scottish humic sites. Reference lines from the y-axis of Figure 15 are used to predict pH values.



The High-Good boundary in Figure 19 represents (or closely matches) breakpoints for functional groups and grazers. However, the non-linear response of many of these metrics makes interpretation difficult. The diversity metrics suggest that the good status band has higher diversity than high status. This may imply that the boundary should be lower, although the response of species to pressure is often not linear and it is a well-established paradigm that under low to moderate levels of disturbance species may increase (e.g. Wilkinson, 1999). There is a noticeable decline in WFD-AWICsp when the pH falls to 4.55, that is, the Good-Moderate boundary. This also occurs for Shannon diversity and number of taxa, indicating an ecological effect at least towards the lower end of good status. The functional groups provide more evidence of a change between good and high status and hence support the boundary location where grazers are noticeably lower in good status and shredders begin to increase. At pH 4.55 there is more clear evidence of community change and an increasingly impacted community. Below this figure WFD-AWICsp, Shannon diversity, NTaxa, functional groups and grazers all decline markedly, providing strong evidence for increasing stress. These declines are mirrored by increases in shredders as well as an increased parasitic loading. These general trends continue into the poor and bad classes.

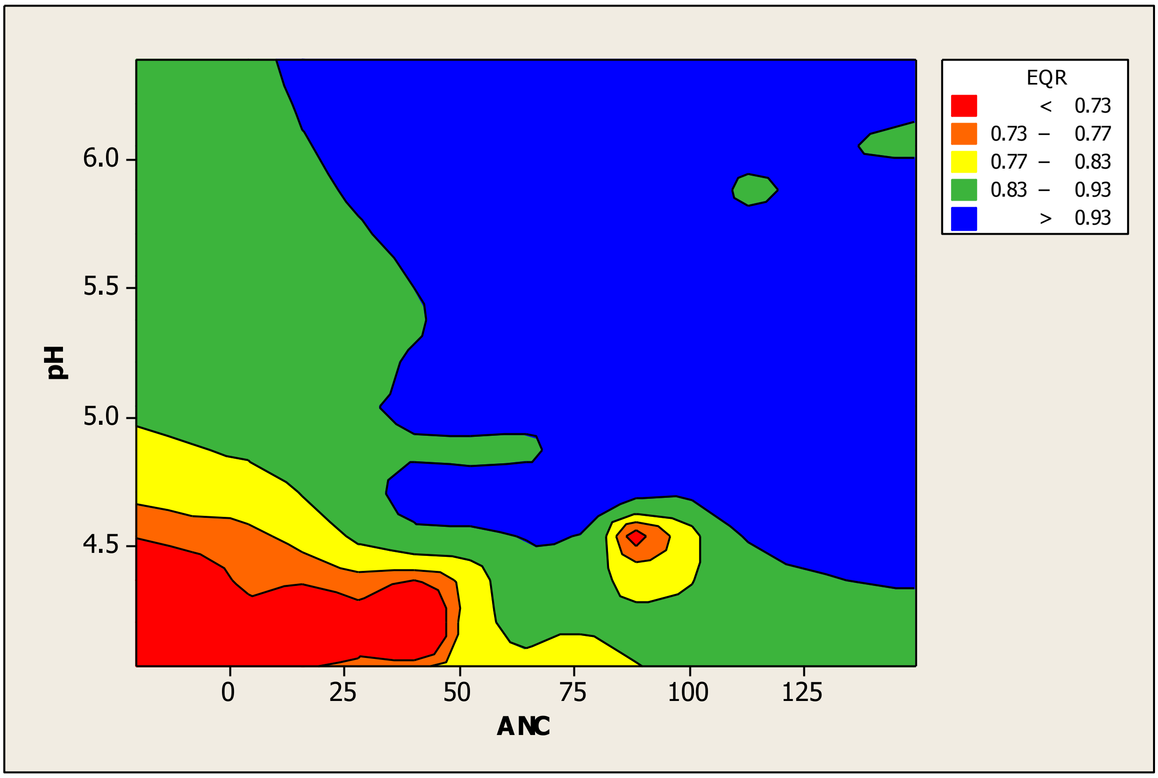
Figure 19: Scatterplots of WFD-AWICsp, diversity metrics and selected functional groups verses pH at Scottish humic sites (>10 mg/l DOC) with Lowess fit. Reference lines are derived from Figure 18.



5.5.3 Combining ANC and pH to predict status

A contour plot can now be constructed using ANC and pH with EQR values overlaid as the Z-variable (Figure 20). Caution needs to be used in the interpretation as the EQR is not independent from ANC and the pH boundaries are then derived from the EQR. However, the plot does enable a useful interpretation of what class might be expected against both ANC and pH. Above ANC 80 µeq/l and pH 5.1 we can expect sites to be usually at high status. However, below ANC 40 µeq/l, even if the pH remains over pH 5.1, we can expect a site to be in good status or lower. Conversely, if a site has an ANC above 80 µeq/l, it has to have a very low pH (<4.5) before we can predict a decline in status class. This supports the view that sites in humic waters can have a very low pH but still be in high status. Below ANC 80 µeq/l and pH 5.1 more sites are below high status, although not until pH 4.5 will a site rarely, if ever, be in high status, irrespective of ANC. Between pH 4.6 and 4.3 at ANC levels 15-40 µeq/l (i.e. where we would expect moderate status sites) there is a rapid, precipitous decline in class from good to bad. This makes deriving moderate boundaries difficult. In this section of the plot moderate status does contribute to the largest area, if only marginally over poor status. Below ANC 15 µeq/l and pH 4.3 only bad status sites are predicted, perhaps suggesting the EQR boundary for poor-bad should be higher.

Figure 20: Contour plot of pH verses ANC with EQR overlaid as the Z-variable at Scottish humic sites (>10 mg/l DOC)



6.0 Boundary Development for Scottish Clear-water Sites

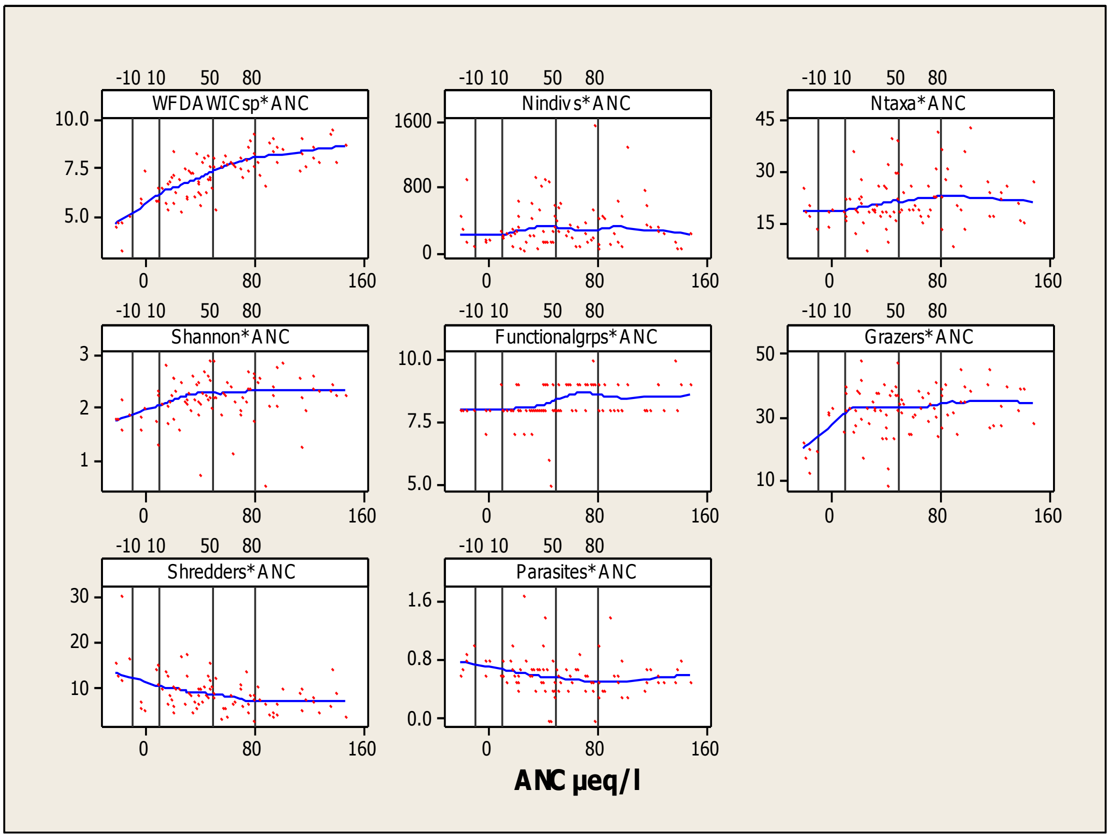
For a site to be included in the boundary development it had to have a minimum of four chemical samples taken in the previous two years from the date of the biological sample. Eighty six sites met these criteria and had DOC <10 mg/l.

In a similar process used for humic sites, potential step-changes in the pressure-response gradient were analysed using Lowess Smoothing (degrees of smoothing = 0.5, steps = 2). The metrics used were the same; WFD-AWICsp, number of individuals, number of taxa, Shannon Diversity, the number of functional groups, grazers, shredders and parasites.

6.1 High-Good Boundary

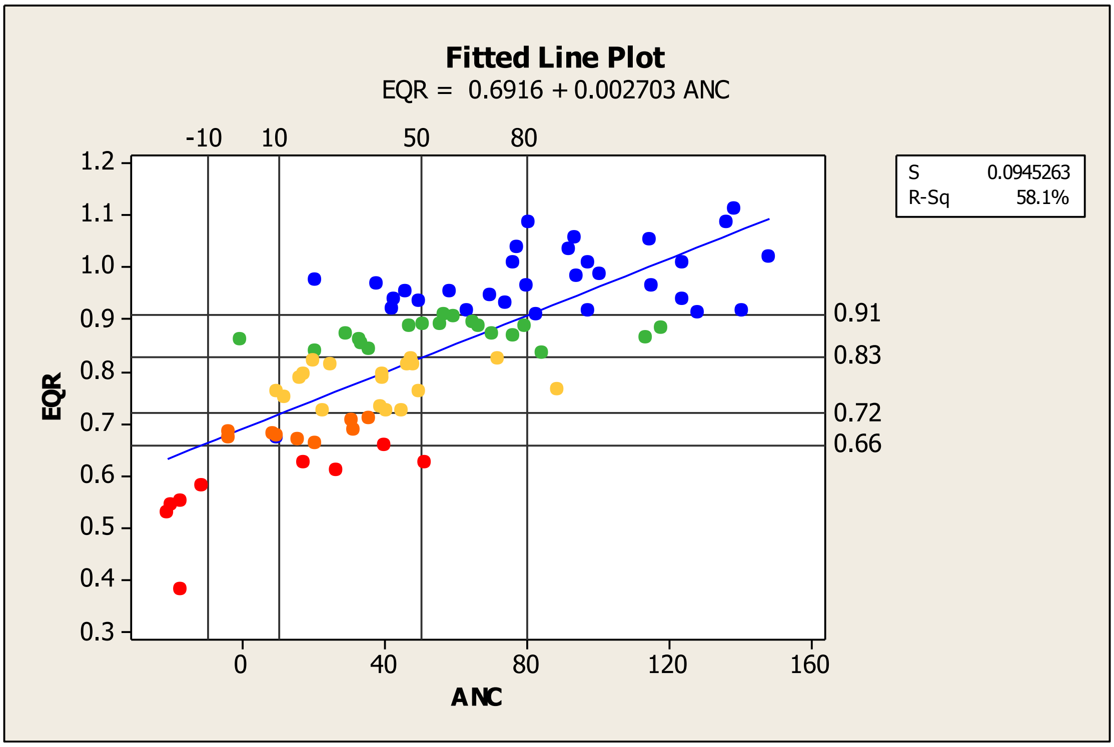
The High-Good boundary was chosen at ANC 80 µeq/l, the same threshold as for humic sites. For further discussion on this threshold refer to the humic water section. Above the 80 µeq/l threshold WFD-AWICsp remains at a relatively constant level and, although there is a gradual decline from 150 to 80 µeq/l, at this threshold WFD-AWICsp is still above 8.0 (Figure 21). This is a high metric score where a sample will have a number of sensitive and highly sensitive taxa present. Below ANC 80 µeq/l the angle of the smoothing line for WFD-AWICsp steepens. At this threshold we also begin to see the first noticeable decline in diversity (NTaxa and Shannon Diversity). Grazers also appear to decline, if only marginally. This is broadly mirrored in the opposite direction by Shredders. As with humic sites, the declining grazer population, coupled with increases in shredders may indicate the shift from autochthonous to allochthonous carbon reflecting changes in ecosystem processes with reduced primary production and external carbon sources becoming increasingly important. This may represent a natural community response to decreases in nutrients within the system, rather than a response to stress from acidification. The parasitic contribution to the community declines from ANC 150 µeq/l to 80 µeq/l, in contrast to the expected response. This is a similar response to that noted in humic waters. For clear waters, however, parasites begin to increase through the good status class, a broadly linear increase through to the most acidified sites. Parasitic loading within a community tends to become greater towards more stressed environments (Lafferty & Kuris,1999) and so it is perhaps surprising that at the high-good boundary we begin to see increases in this functional group, suggesting perhaps the boundary should be set higher. However, this is not reflected by the two other functional groups. Neither grazers or shredders provide evidence that the boundary should be higher than 80 µeq/l

Figure 21: Scatterplots of WFD-AWICsp, diversity metrics and selected functional groups verses ANC, at Scottish clear-water sites (DOC <10 mg/l), with Lowess fit. Breakpoints are represented by reference lines along the x-axis.



The threshold figure of 80 µeq/l can be applied to a linear regression equation between WFD-AWICsp EQRs and ANC (Figure 22). Using the x-axis as the predictor results in a High-Good boundary of 0.91. The regression is highly significant with a high R^2 (R^2 = 0.58, P = <0.001) and so we can be confident that 0.91 is ecologically relevant in relation to the scatterplots.

Figure 22: Linear regression of WFD-AWICsp EQRs verses ANC at Scottish clear-water sites (DOC <10 mg/l). Reference lines are derived from Figure 21 and are used to predict EQR boundaries on the y-axis. (● = High, ● = Good, ● = Moderate, ● = Poor, ● = Bad).



6.2 Good-Moderate Boundary

The Good-Moderate boundary was chosen at ANC 50 µeq/l. Again this may appear rather high, the UCL damage matrix (Monteith and Simpson, 2007) classifies sites at ANC 50 µeq/l as either high or good status depending on calcium levels. However, episodic acid events common in rivers may mean that actual lower ANC values are considerably less than this and so the ANC figure for community change from good to moderate status is likely to be much lower.

At the 50 µeq/l threshold the smoothing line for WFD-AWICsp is some way down a continuous decline to the most acidified sites. NTaxa continues to decline indicating the presence of fewer highly sensitive and sensitive taxa (Figure 21). A drop in Shannon diversity is also noted, if only marginal. A breakpoint in the Lowess fit for Shannon diversity does not occur until ANC 40 µeq/l, suggesting perhaps this figure may be better for the Good-Moderate boundary. This would be in closer agreement with the UCL damage matrix and also the use of 40 µeq/l for humic waters. However, for clear-water sites WFD-AWICsp begins falling at a higher ANC and both shredders and parasites are increasing at this point. The line for grazers declines only very slightly and remains unchanged until 10 µeq/l, differing to humic waters. We might conclude from this that widespread functional change does not occur until lower ANC values: in other words, at more stressed sites than those at the Good-Moderate boundary.

6.3 Moderate-Poor Boundary

The moderate-poor boundary was set using ANC 10 µeq/l. At this level the WFD-AWICsp continues to decline with the loss of the most sensitive taxa. The UCL damage matrix generally classifies sites at this level of ANC as either moderate or poor. Below 10 µeq/l the line steepens suggesting increased response to pressure. The Lowess line for NTaxa and number of functional groups levels out. Generally most of the sensitive and highly sensitive taxa have been lost at this threshold and so diversity as measured by simple numbers of taxa remains unchanged. However, the decline in Shannon diversity is due to lower evenness in the community. Sites in poor status, whilst often having similar taxa as moderate sites, are characterised by higher numbers of tolerant taxa and fewer, less abundant, moderately sensitive species. Just above 10 µeq/l there is a distinct breakpoint in grazers, with a rapid decline to the most acidified sites. This suggests that at this threshold large changes in community function occur. Shredders and parasites continue to increase supporting this view.

6.4 Poor-Bad Boundary

The Poor-Bad boundary has been set at ANC -10 µeq/l resulting in an EQR of 0.66. There are few data to determine the location of the boundary here and so caution should be applied to this. Using a threshold of -10 µeq/l, the UCL damage matrix classifies sites at this level of ANC as either bad or poor. The use of this threshold is supported by the very low WFD-AWICsp for the nine sites below the threshold. Only three of these sites have any moderately sensitive species (two sites have *E.aenea* and one site has *L.volckmari*). The rest of the community consists of tolerant and highly tolerant taxa only, often in high abundances. The decline in Shannon diversity continues whilst grazers continue to fall with a corresponding increase in shredders and parasites.

6.5 Boundary Evaluation

6.5.1 Contour Plots

For a further evaluation of the location of the boundaries, contour plots were used (distance method, power = 2) where the Z variables are for significant metrics (i.e. those used in Figure 21) v pressure, using linear regression, after testing for normality using Ryan-Joiner, Johnson’s transformation where required and Bonferroni correction (significance where P = <0.006).

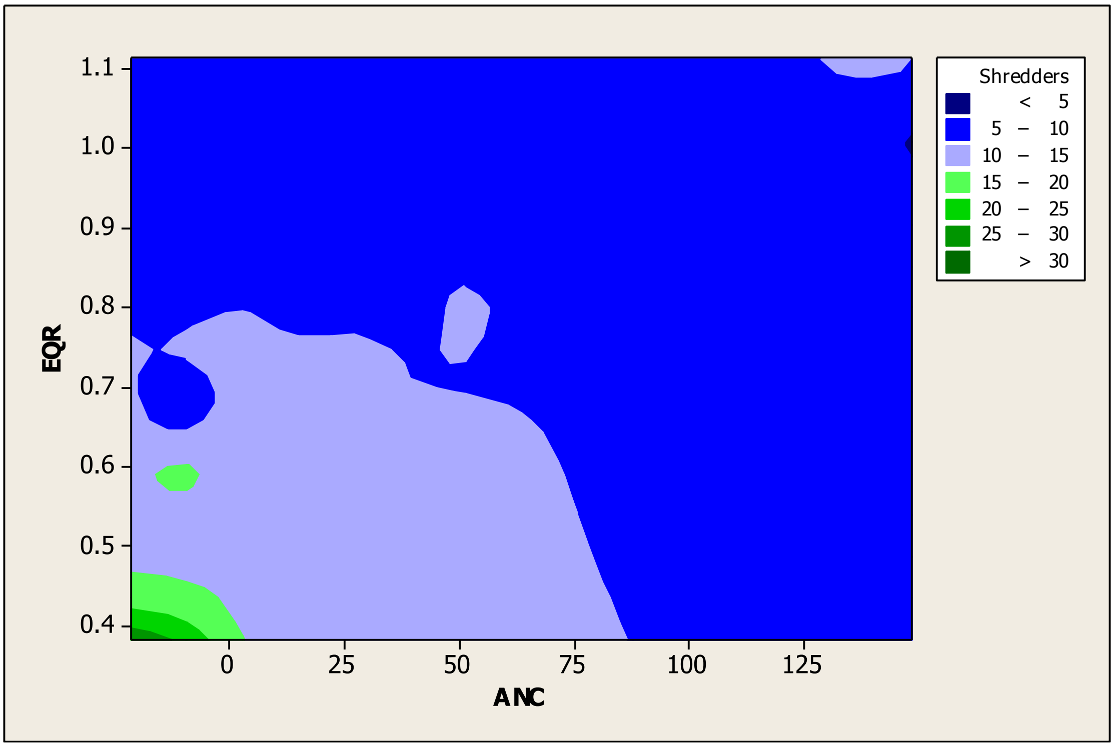
The functional group shredders, was the only metric (excepting WFD-AWICsp) found to respond significantly to ANC. Shredders (Table 19 and Figure 23) are found at good and high status sites at typically <10%. This can increase up to 15% at moderate status sites. Below ANC 10 µeq/l and EQR 0.72 (i.e. below the Moderate-Poor boundary) sites will invariably have shredders >10% and increasingly well over 15%. This is mirrored by a fall in grazers to <30%. Rapid increases in shredders can be seen below ANC 0 µeq/l.

Table 19:Typical percentage shredder contributions to classes for Scottish clear-water sites (<10 mg/l DOC). Grazers are shown for comparison.

Status Class	Grazers (Typical % Contribution)	Shredders (Typical % Contribution)
High	30-40%	5-10%
Good	30-40%	5-10%
Moderate	25-35%	5-15%
Poor	25-35%	10-15%

Bad	<25%	10-30%

Figure 23: Contour plot of clear-water EQR verses ANC with percentage shredders overlaid as the Z-variable.

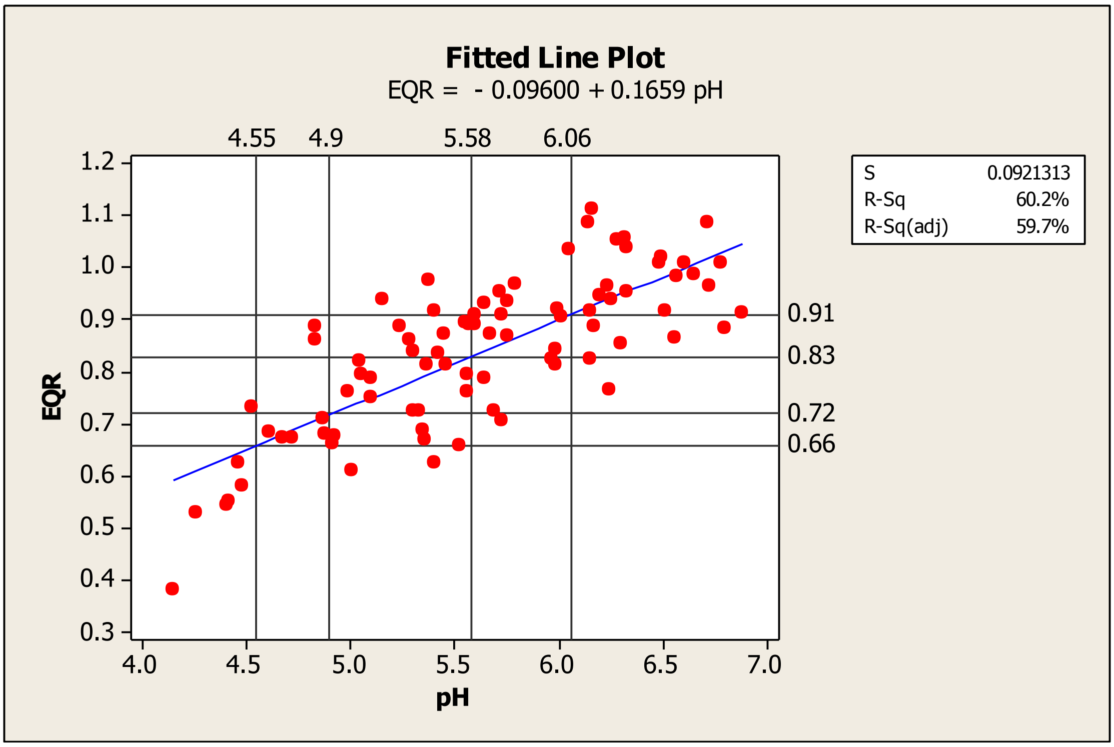


6.5.2 Predicted pH boundaries

As a further evaluation of the validity of the boundaries, EQR values have been used to predict pH values from a linear regression (Figure 24). The relationship is highly significant (R^2 = 0.6, *P = <0.001*) and compares favourably with the regression with ANC (R^2 = 0.58, *P = <0.001*). An interesting pattern noted during these analyses is that pH rarely performs as well as ANC as a predictor for an acid metric response when a wide gradient of DOC is used. pH is generally highly related to DOC but pressure metrics exhibit a non-linear response to DOC especially if the gradient goes >10 mg/l. This is because at these high levels DOC (i.e. humic acids) acts as an effective buffer to toxicity and so sensitive taxa can exist in lower pH waters than might be expected at lower DOC sites. At higher DOC levels there is a divergence of metric values away from the regression line, as seen in Figure 2. In other words, pH becomes a poorer predictor of metric scores, and hence class, in high DOC, low pH waters, compared to ANC. However, when the DOC gradient is split into a typology, then pH becomes a better predictor, especially in low DOC waters. This buffering can also clearly be seen from the resulting boundaries which result in pH values as 6.06 (High-Good), 5.58 (Good-Moderate), 4.9 (Moderate-Poor) and 4.55 (Poor-Bad). These are much higher, as expected, than the pH values derived from the humic sites (5.1, 4.55, 4.22, 4.03) and relatively much larger than the differences between the ANC scores from the two typologies (humic rivers; 80, 40, 15, 0 clear rivers; 80, 50, 10, -10).

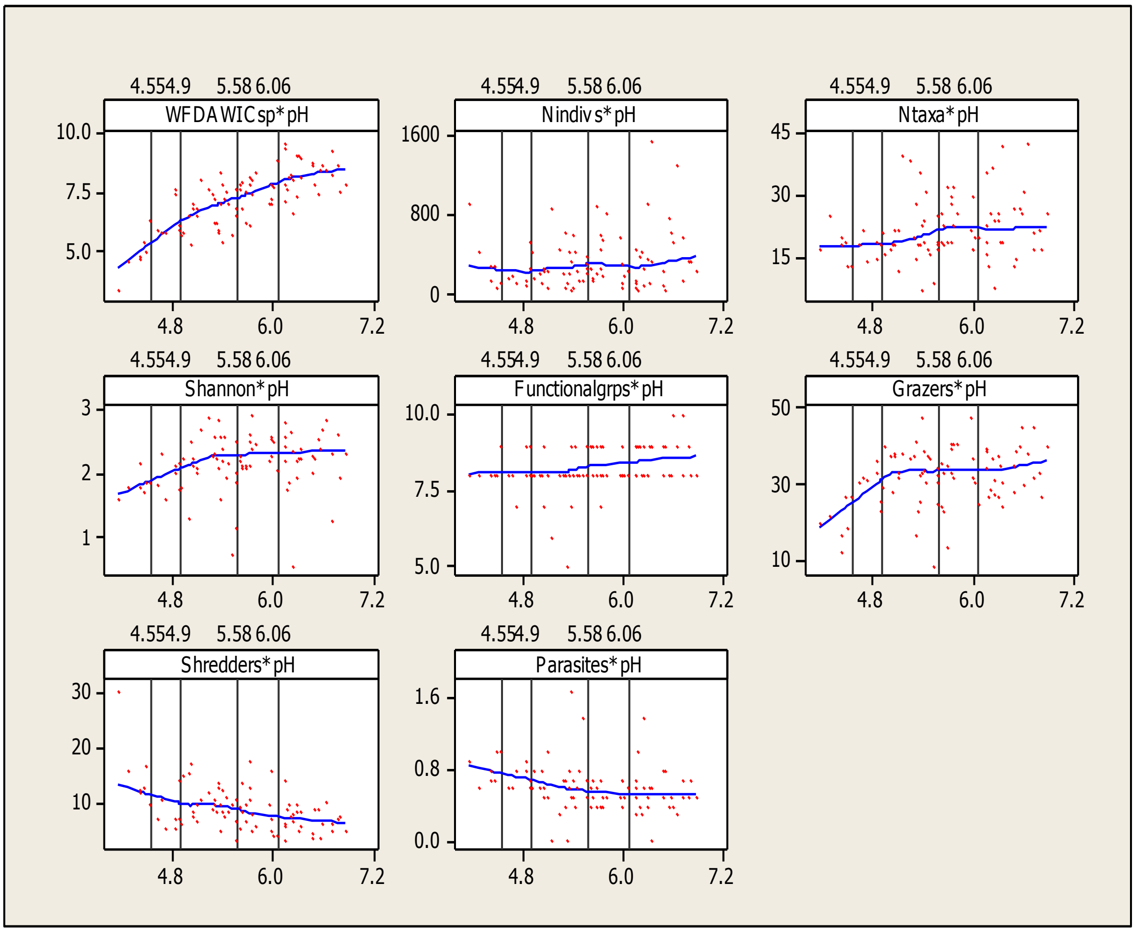
The values can be compared to the same set of metrics used to develop boundaries using ANC and step-changes can again be investigated using Lowess smoothing (degrees of smoothing = 0.5, steps = 2).

Figure 24: Linear regression of WFD-AWICsp verses pH at Scottish clear-water sites (<10 mg/l DOC). Reference lines from the y-axis of Figure 22 are used to predict pH values.



The High-Good boundary in Figure 25 represents a breakpoint where the line steepens for WFD-AWICsp, supporting 0.91 as a boundary. There is no obvious response from NTaxa or individuals although the slight increases in both may suggest low levels of disturbance. There are no clear breakpoints in Shannon diversity or grazers until further down the pH gradient, suggesting significant change in function and falling diversity does not occur at good status. However, there is a continued increase in the percentage of shredders and a gradual fall in the number of functional groups. This may suggest a small shift in resource processing between the high and good status classes. From pH 6.06 to pH 5 WFD-AWICsp continues a linear decline making it difficult to set the good-moderate boundary. However, at pH 5.58 there is a breakpoint in the number of taxa and number of functional groups. This suggests that the Good-Moderate boundary of 0.83 does represent a decline in diversity and functional complexity. Additionally, the Lowess Fit for parasites steepens, suggesting increased stress. A distinct breakpoint in Shannon diversity does not occur until pH 5.3, in the middle of moderate class. This might imply the EQR should be lower. However, this would mean that good status class would have substantially lower levels of taxa and WFD-AWICsp compared to high status. At pH 4.9, representing the moderate-poor boundary the smoother for WFD-AWICsp becomes steeper supporting the EQR of 0.72. This is also marked by the sharp decline in grazers and an increase in shredders. This EQR therefore supports distinct functional change. Below this point there is little decline in number of taxa, although Shannon continues to fall. This indicates the loss of sensitive taxa has already occurred but falling eveness continues, with increased dominance of tolerant and highly tolerant species. Below pH 4.9, WFD-AWICsp, Shannon, grazers, shredders and parasites all decline or increase in a consistent linear response making an evaluation of the Poor-Bad boundary difficult. This was also the case for the boundary setting using ANC and so a certain amount of caution needs to be applied when using this boundary.

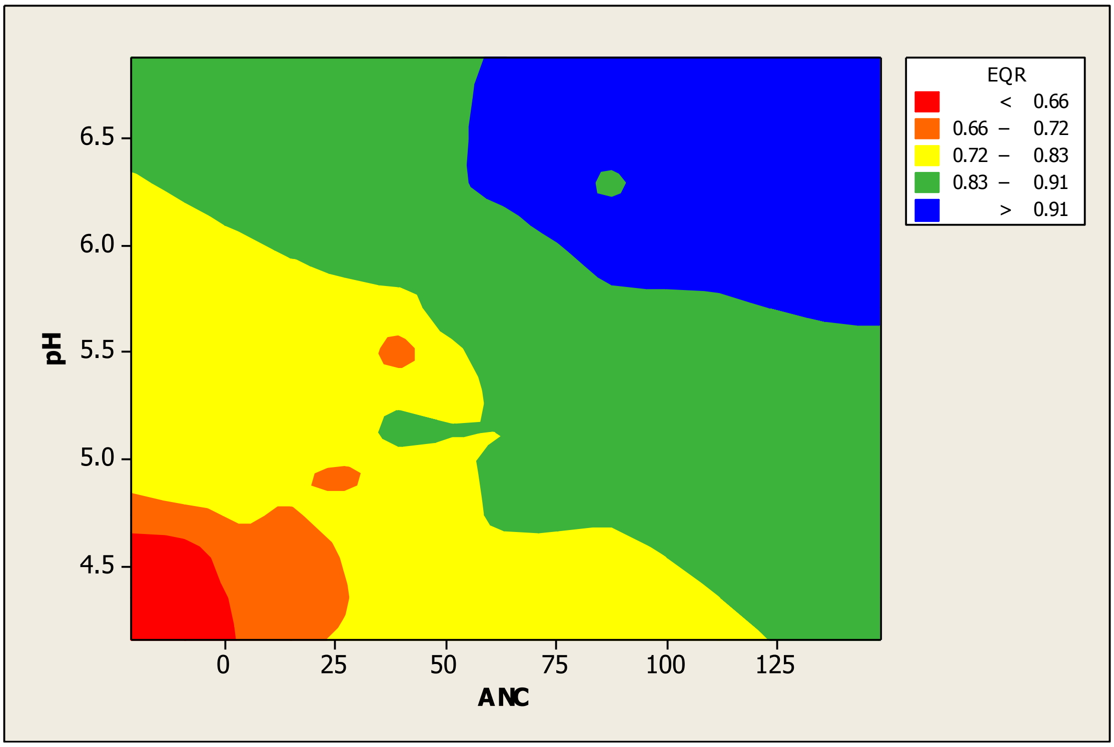
Figure 25: Scatterplots of WFD-AWICsp, diversity metrics and selected functional groups verses pH at Scottish clear-water sites (<10 mg/l DOC) with Lowess fit. Reference lines are derived from Figure 24.



6.5.3 Combining ANC and pH to predict status

A contour plot can now be constructed using ANC and pH, the resulting predicted EQR values overlaid as the Z-variable (Figure 26). Caution needs to be used in the interpretation as the EQR is not independent from ANC and the pH boundaries are then derived from the EQR. However, the plot does enable a useful interpretation of what class might be expected against both ANC and pH. Between ANC 50 and 0 µeq/l, if the pH remains over pH 6.06, a site is expected to be in good status. Below ANC 0 µeq/l a site is likely to be at moderate status, even if the pH is > 6.06. Humic sites with ANC levels above 80 µeq/l, have to have a very low pH (<4.5) before we can predict a decline in status class. As expected, clear-water sites can have a much higher pH when predicting a decline in status class (5.6) due to lower buffering from humic acids. In clear waters below pH 5.6, a site will rarely, if ever, be in high status, irrespective of ANC. Between pH 5.58 and 4.9 at ANC levels 10-50 µeq/l (i.e. where we would expect moderate status sites) sites are generally classified as moderate. At these pH levels, above ANC 50 µeq/l a site is much more likely to be at good status. Between pH 4.55 and 4.9 the ANC would have to be >20 µeq/l before moderate status is more likely and > 58 µeq/l for good status. Below ANC -10 µeq/l and pH 4.55 the status will be invariably bad.

Figure 26: Contour plot of pH verses ANC with EQR overlaid as the Z-variable at Scottish clear-water sites (<10 mg/l DOC).



7.0 Boundary Development for Welsh Sites

For a site to be included in the boundary development it had to have a minimum of four chemical samples taken in the previous two years from the date of the biological sample. One hundred and thirty nine sites met these criteria.

In a similar process to that used for Scottish sites, potential step-changes in the pressure-response gradient were analysed using Lowess Smoothing (degrees of smoothing = 0.5, steps = 2). The metrics used were the same; WFD-AWICsp, number of individuals, number of taxa, Shannon diversity, the number of functional groups, grazers, shredders and parasites (Figure 27).

7.1 High-Good Boundary

The High-Good boundary was chosen at ANC 80 µeq/l, the same as for Scottish sites. The threshold figure of 80 µeq/l can be applied to a linear regression equation between WFD-AWICsp EQRs and ANC (Figure 28). Using the x-axis as the predictor results in a

High-Good boundary of 1.00. The regression is highly significant with a high R^2 ($R^2 = 0.63$, P = <0.001) and so we can be confident that 1.00 is ecologically relevant in relation to the scatterplots. Above the 80 µeq/l threshold, in contrast to Scottish humic sites, WFD-AWICsp is still rising. This is also the case for some of the other metrics e.g. NTaxa, Shannon diversity and number of functional groups, contrasting to Scottish clear-water rivers. This may suggest the high-good boundary should be set higher. However, given the boundary is 1.0, any EQR set higher would be counter-intuitive, although this may suggest the reference value is too low for Welsh sites. Indeed, the reference value of 7.65 for Welsh sites is noticeably lower than that for Scottish clear-water sites (8.61). The use of a high-good boundary for Welsh sites at 1.0, compared to 0.91 for Scottish sites, reduces this difference in terms of the raw metric value score at the high-good boundary (Wales = 7.65, Scotland = 7.85). The Lowess smoother is based on few data above 80 µeq/l and so the slope of the line needs to be treated with caution. Neither grazers or shredders appear be responding above this threshold, contrasting to Scottish humic sites. In the light of this, 80 µeq/l appears to be a reasonable threshold to take, albeit with some uncertainty.

7.2 Good-Moderate Boundary

The Good-Moderate boundary was chosen at ANC 40 µeq/l. At the 40 µeq/l threshold the smoothing line for WFD-AWICsp is some way down a continuous decline to the most acidified sites. A step-change is noticeable at ANC 20 µeq/l and this may suggest this would be a better location for the Good-Moderate boundary. However, episodic acid events common in rivers may mean that actual lower ANC values are considerably less than those represented by the data and so the ANC figure for community change from good to moderate status is likely to be much lower in reality than 40 µeq/l. Additionally, several of the other metrics support the 40 µeq/l threshold. The slope for NTaxa has declined substantially by this point and the breakpoint at 40 µeq/l suggests the loss of most of the sensitive taxa. The number of functional groups also follows this pattern. This threshold marks a distinct breakpoint and decline in the percentage of grazers. Whilst not reflected by shredders (which don't increase until ANC 5 µeq/l), it may represent observable functional change.

Figure 27: Scatterplots of WFD-AWICsp, diversity metrics and selected functional groups verses ANC, at Welsh sites, with Lowess fit. Breakpoints are represented by references lines along the x-axis.

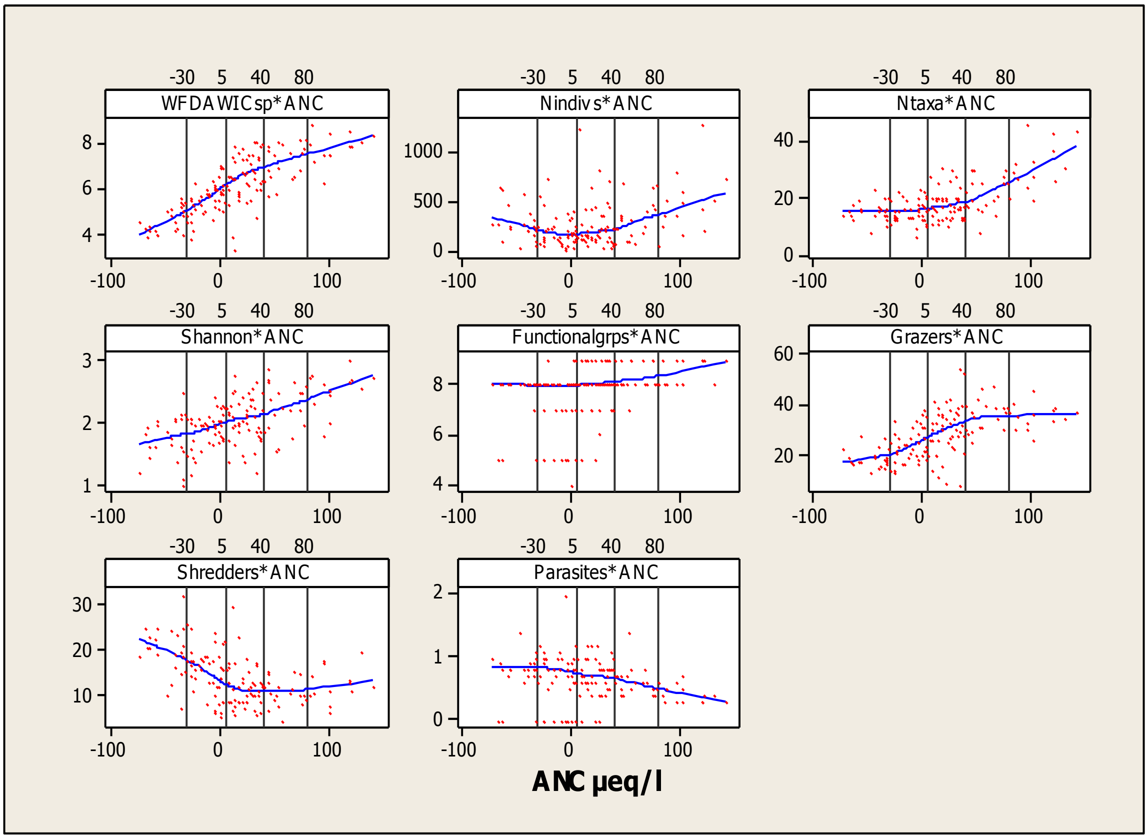
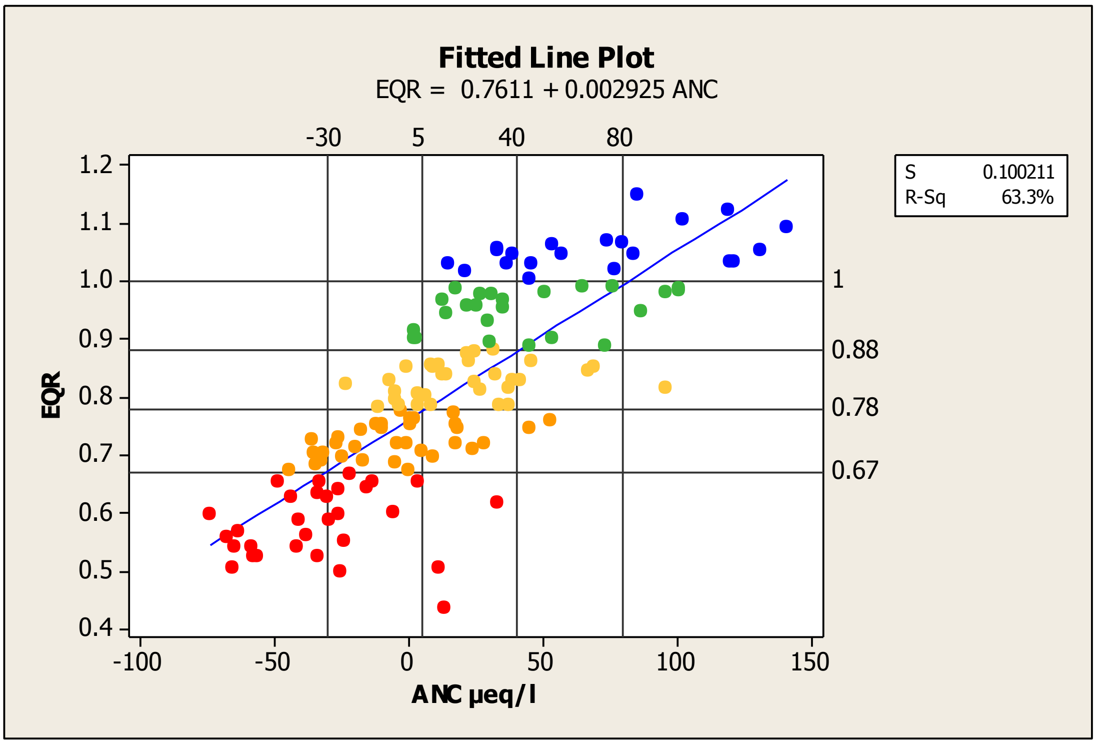


Figure 28: Linear regression of WFD-AWICsp EQRs verses ANC at Welsh sites. References lines are derived from Figure 27 and are used to predict EQR boundaries on the y-axis.



7.3 Moderate-Poor Boundary

The moderate-poor boundary was set using ANC 5 µeq/l. At this level the WFD-AWICsp continues to decline and the UCL damage matrix classifies all sites at this level of ANC as either moderate or poor. The Lowess line for number of taxa levels out suggesting all sensitive and highly sensitive taxa have been lost at this threshold. Shannon diversity continues to decline due to lower evenness in community structure. Sites in poor status, whilst often having similar taxa as moderate sites, are characterised by higher number of tolerant taxa and fewer, less abundant, moderately sensitive species. These trends are broadly similar to the Moderate-Poor boundary for Scotland. At this threshold shredders begin to increase rapidly suggesting large changes in community function.

7.4 Poor-Bad Boundary

The Poor-Bad boundary has been set at ANC -30 µeq/l resulting in an EQR of 0.67. This appears a very low figure and using a threshold of -30 µeq/l, the UCL damage matrix classifies all sites as bad. However, at this point the Lowess line continues to fall for WFD-AWICsp, Shannon and grazers, with a corresponding increase in shredders. Interestingly, the number of individuals increase at these very low ANC levels. This is due to very high abundances at some sites of a few tolerant and highly tolerant species. This may either indicate increased niche utilisation of these species at these highly stressed sites, or predator release, or perhaps both.

7.5 Boundary Evaluation

7.5.1 Contour Plots

For a further evaluation of the location of the boundaries contour plots were used (distance method, power = 2) where the Z variables are for the significant metrics (i.e. those used in Figure 27) v pressure, using linear regression, after testing for normality using Ryan-Joiner, Johnson's transformation where appropriate, and Bonferroni correction (significance where *P* = <0.006).

In contrast to the Scottish data most of the metrics were found to respond significantly to ANC. For the purposes of this report the three with the highest R^2 are illustrated and discussed; NTaxa, Shannon diversity and grazers. Table 20 and Figure 29 indicate that around ANC 80 µeq/l NTaxa increases rapidly. At a High-Good boundary of 1.0 we can expect a sample to have >25 taxa, although most sites over >80 µeq/l can expect to have >25 taxa down to EQR 0.94. This may suggest that the High-Good boundary is too high. However, this is not supported by Shannon diversity which suggests most sites will have clearly lower diversity by this point (Figure 30). Good status sites typically have 20-25 taxa. Below 40 µeq/l, in the good band (i.e. 0.88-1.00), sites with <20 taxa become more common. This supports the position of the good status class. Both moderate and poor status classes typically have between 15-20 taxa, although at poor status (EQR 0.67-0.78, ANC -30-5 µeq/l), <15 taxa in samples begin to appear. At bad status 15-20 taxa is still the most common category, although sites with <15 taxa become more frequent. For Shannon diversity, high status sites typically have scores between 2.25-2.75, indicating large numbers of taxa and high evenness. This decline through the classes is relatively consistent through to bad status. Although sites at bad status can have Shannon diversity scores up to 2.00, some can go below 1.5, indicating a very poor sample. It should be noted that low numbers of taxa (<20) and low Shannon diversity (<2.00) can occur almost all the way along the ANC gradient. This many indicate other pressures affecting these metrics at some sites, although the chances of getting a low Shannon diversity score declines rapidly above 50µeq/l. For grazers, high status sites will typically have >30% (Figure 31). However, higher percentages (>40%) can be expected above EQR 0.97, if the ANC is above 25 µeq/l. These contours are influenced by a couple of sites at the upper end of good status with very high grazer numbers. The Lowess smoother in Figure 28 suggests overall grazers begin to decline at 40 µeq/l. The evidence suggests that there is no difference between high and good status for grazers. From moderate through to bad status there is a noticeable decline.

Table 20:Typical number of taxa, Shannon Diversity and percentage grazer contributions to classes for Welsh sites

Status Class	Number of Taxa (Typical number)	Shannon Diversity (Typical Score)	Grazers (Typical % Contribution)
High	>25	2.25-2.75	>30%
Good	20-25	2.00-2.50	>30%
Moderate	15-20	1.75-2.25	25-35%
Poor	10-20	1.75-2.00	20-30%
Bad	10-20	<1.50-2.00	15-25%

Figure 29: Contour plot of EQR verses ANC for Welsh sites with number of taxa overlaid as the Z-variable.

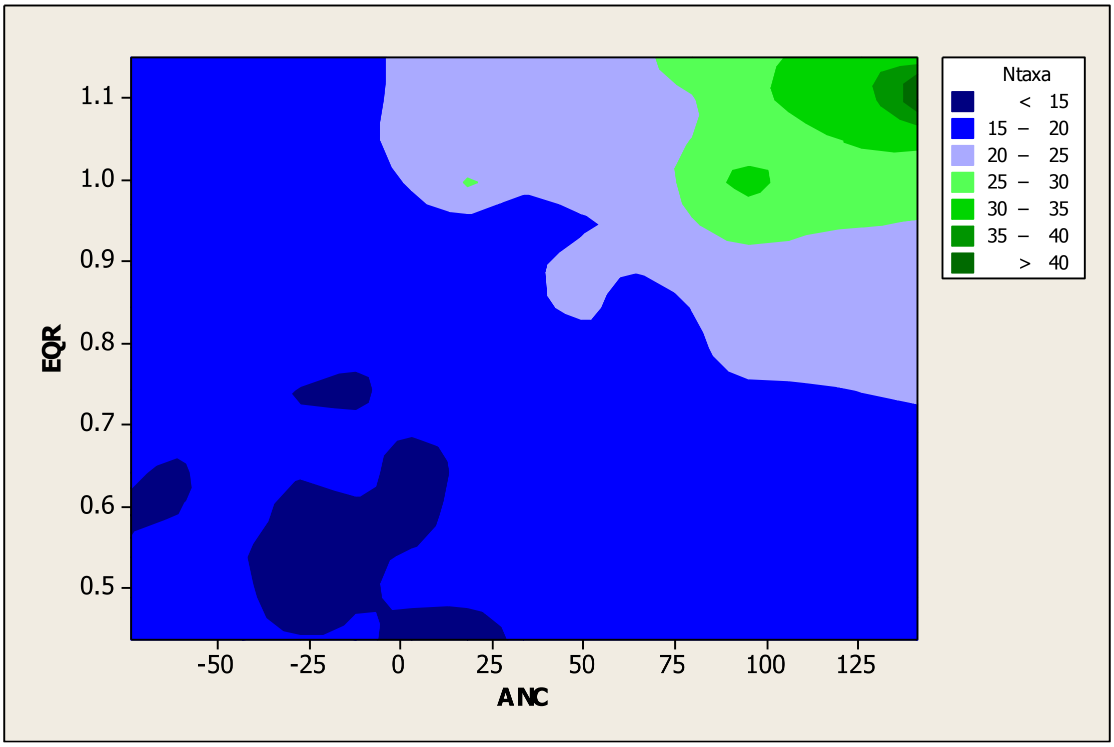


Figure 30: Contour plot of EQR verses ANC for Welsh sites with Shannon diversity overlaid as the Z-variable.

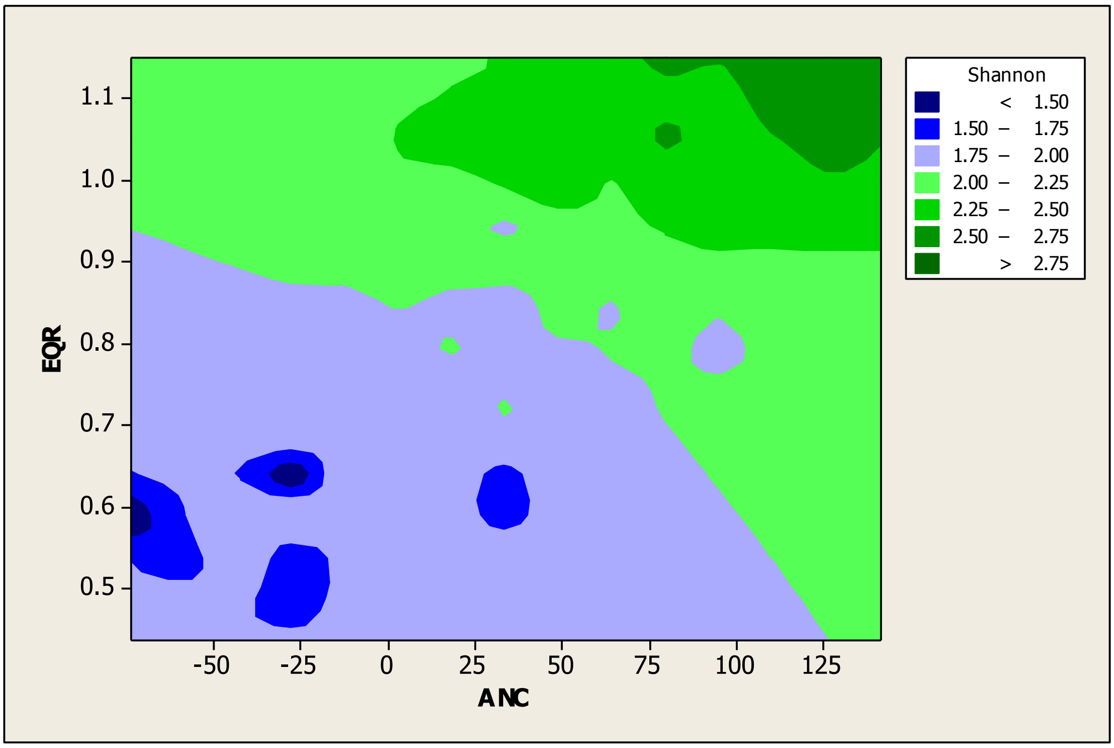
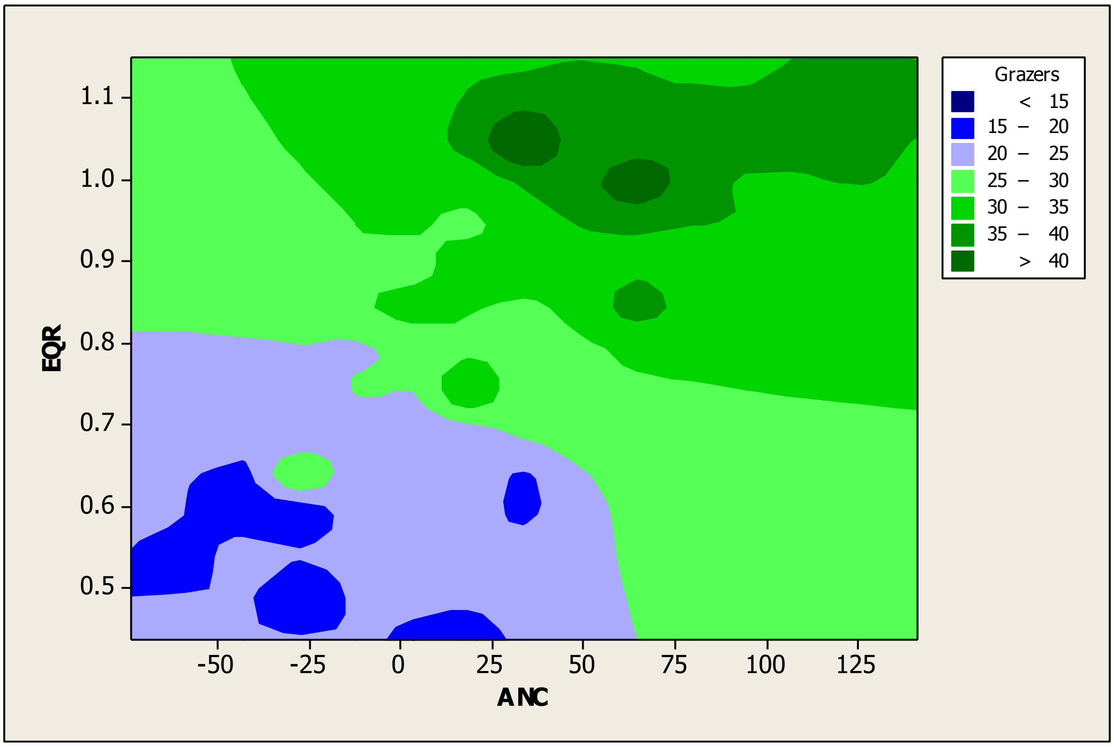


Figure 31: Contour plot of EQR verses ANC for Welsh sites with percentage grazers overlaid as the Z-variable.



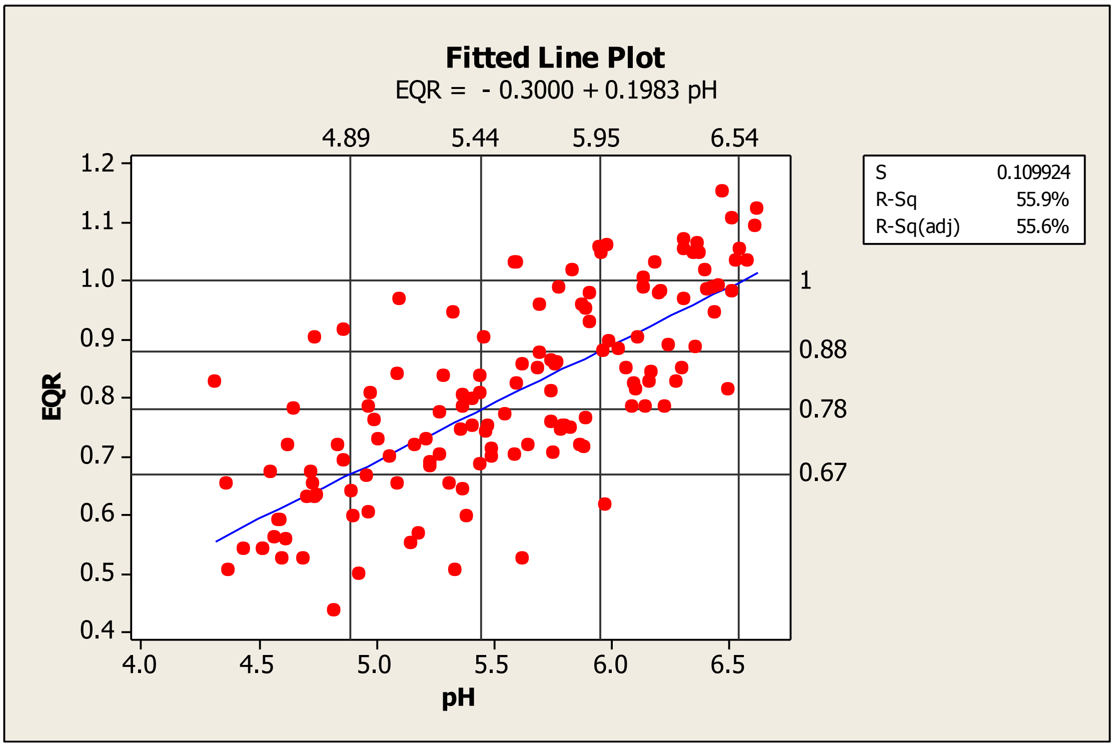
7.5.2 Predicted pH boundaries

As a further evaluation of the validity of the boundaries EQR values are used to predict pH values from a linear regression (Figure 32). The relationship is highly significant (R^2

= 0.56, *p* = <*0.001*) although it is less than the regression with ANC (R^2 = 0.63, *p* = <*0.001*). As commented in the Scottish clear-water section, pH rarely performs as well as ANC as a predictor for an acid metric response when a wide gradient of DOC is used. For Welsh lakes there is no typology based on DOC. This is because there were few high DOC sites. The vast majority of Welsh sites have DOC < 5mg/l, with the highest recorded DOC being 9.7 mg/l and a mean value of 2.89 mg/l. Therefore, it is perhaps surprising that the fit between EQR and pH is not reflected by an R^2 value comparable with that for ANC. In contrast to Welsh sites, the mean DOC for Scottish clear-water rivers is much higher (5.52 mg/l). Although at Scottish sites DOC was found to only make a significant difference in reference communities >10 mg/l at pH below 5.5, the higher mean DOC even in Scottish clear-water rivers may generally provide sites in Scotland with more buffering against low pH than many Welsh rivers. We might conclude DOC will have little relevance for Welsh data and this may account for the resulting pH values of 6.54 (High-Good), 5.95 (Good-Moderate), 5.44 (Moderate-Poor) and 4.89 (Poor-Bad) being higher than for the Scottish clear-water sites (6.06, 5.58, 4.9 and 4.55), despite the datasets having very similar mean pH values (Scotland = 5.6, Wales = 5.57). In other words, the low DOC and therefore buffering in Welsh rivers, means the macroinvertebrate community is impacted by acidification at a higher pH than for Scottish waters. This contrasts with the ANC which generally have very similar values (the exception being the poor-bad boundary).

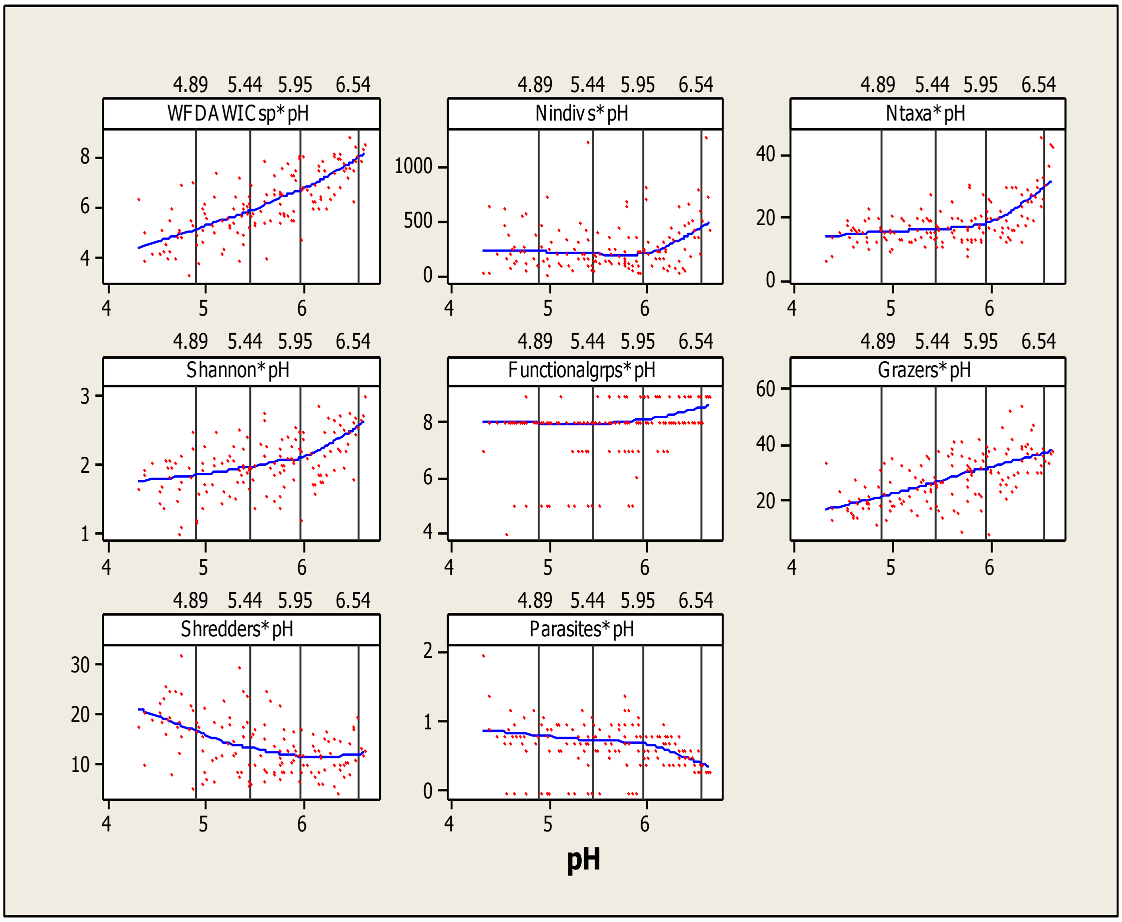
The pH values from Figure 32 can then be compared to the same set of metrics used to develop boundaries using ANC and step-changes can again be investigated using Lowess smoothing (degrees of smoothing = 0.5, steps = 2).

Figure 32: Linear regression of WFD-AWICsp verses pH at Welsh sites. Reference lines from the y-axis of Figure 28 are used to predict pH values.



The High-Good boundary in Figure 32 represents a threshold at the uppermost end of the x-axis. Consequently there is little evidence of any community change at this point, very few sites having pH above this value. It would be hard, therefore, to justify a High-Good boundary set at EQR 1.00. None of the metrics used in Figure 27 or Figure 33 indicate any distinct breakpoints until the Good-Moderate boundary. However, based on evidence from McFarland *et al* (2009), passive filter feeders and xylophagus taxa are functional groups that decline with acidification. Both these two groups indicate breakpoints at ANC 75 µeq/l (Figure 34). Whilst clearly 75 µeq/l is not much lower than 80 µeq/l, using the regression equation (Figure 28) results in an EQR of 0.98. Intuitively this makes more sense for a High-Good boundary than 1.00. Using the equation in Figure 32 results in a pH of 6.45. WFD-AWICsp and the diversity metrics decline fairly steeply through the good class. Grazers indicate a near linear decline throughout the pressure gradient. However, there is no evidence of any increases in shredders until pH 5.95, supporting the Good-Moderate boundary.

Figure 33: Scatterplots of WFD-AWICsp, diversity metrics and selected functional groups verses pH at Welsh sites with Lowess fit. Reference lines are derived from Figure 32.



7.5.3 Combining ANC and pH to predict status

A contour plot can now be constructed using ANC and pH, the resulting predicted EQR values overlaid as the Z-variable (Figure 35). Caution needs to be used in the interpretation as the EQR is not independent from ANC and the pH boundaries are then derived from the EQR. However, the plot does enable a useful interpretation of what class might be expected against both ANC and pH. Above ANC 75 µeq/l and pH 6.45 a site is most likely to be at high status and between ANC 15 and 75 µeq/l, if the pH is >6.45, we can expect a site to be at good status. Below ANC 15 µeq/l a site is likely to be at moderate status, even if the pH is >6.45. As expected, a site should be in good status between ANC 40-75 µeq/l and pH 5.95-6.45. Although sites are more likely to be moderate <40 µeq/l it is not until 15 µeq/l that moderate status actually becomes the dominant class. Moderate status is predicted right across the pH and ANC gradient. This is contrasting to Scotland where it is good status that is predicted across the entire pressure gradient. In the case of Wales, it is unlikely sites will be in moderate status at the upper end of the ANC gradient, although uncertainty relating to episodic events make this perhaps possible. For example, Llwydcoed has been classified as moderate despite an ANC of 95 µeq/l, although out of the 16 sites with ANC >75µeq/l this is the only site classified as less than good.

Figure 34: Scatterplots of passive filter feeders and xylophagus taxa verses ANC, at Welsh sites, with Lowess fit. Breakpoints are represented by references lines along the x-axis.

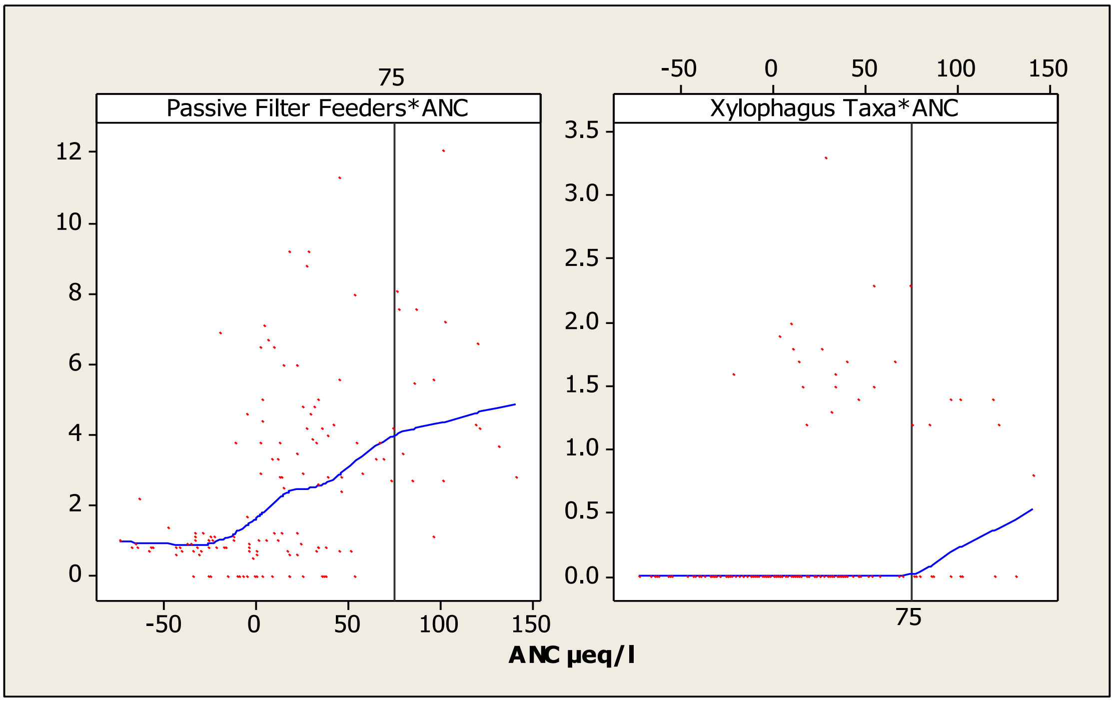
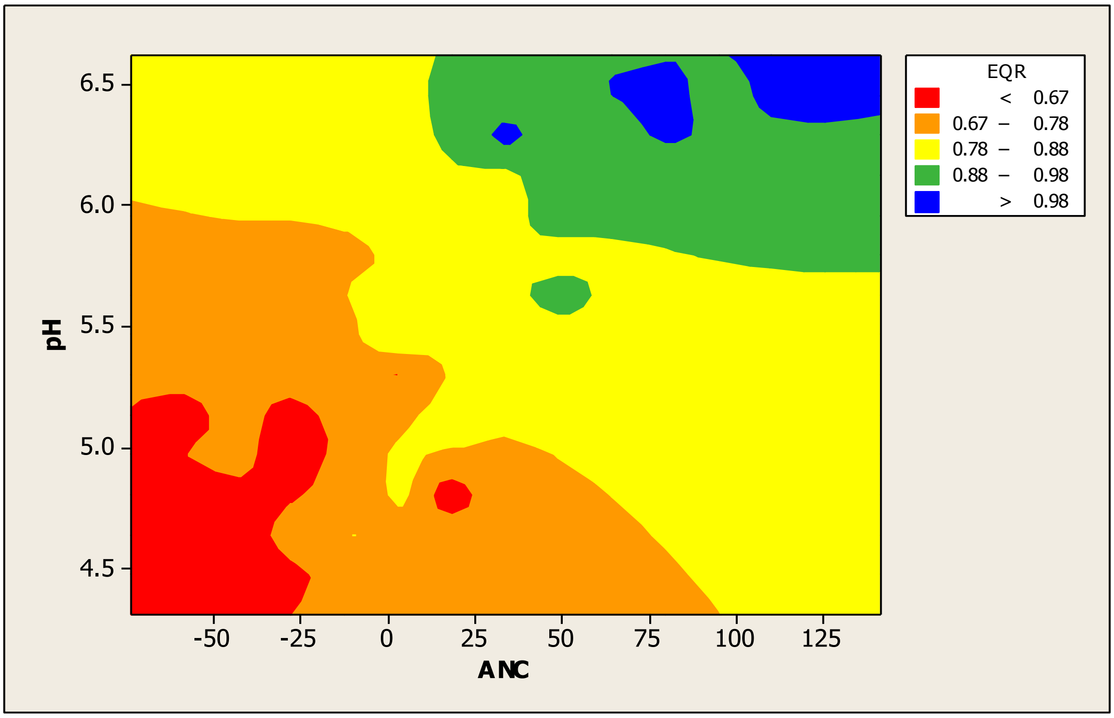


Figure 35: Contour plot of pH verses ANC with EQR overlaid as the Z-variable at Welsh sites.



8.0 Multivariate evaluation of community change between status classes

The derivation of boundaries used univariate thresholds based on the ANC pressure gradient and then compared these to modelled pH thresholds. This section assesses the classified sites in relation to multivariate diversity, function and community data. All analyses in this section were performed in PRIMER 6.

8.1 Diversity

To maintain as much independence as possible (there were not enough data to test on a second independent dataset) a different set of metrics has been used. For assessment of diversity the number of taxa, number of individuals, Shannon diversity and number of functional groups have been replaced by Caswell, Margalef and Pielou diversity metrics.

Principal Components Analysis (PCA) was used to assess diversity, with the diversity metrics treated as environmental variables. Caswell's neutral model (Caswell, 1976) compares the observed fauna with a theoretical expected diversity, given the number of individuals and species present. This gives a *V* statistic, a zero value indicating neutrality with positive and negative values indicating greater or lesser than expected diversity respectively. Margalef's index *(d)* incorporates the total number of individuals and is a measure of the number of species present for a given number of individuals. Pielou's evenness index compares the observed sample with a sample where all taxa were equally abundant (i.e. highest possible evenness). For the purposes of the PCA, all site scores were standardised on a scale of 0-1 to remove negative values. The scores were then square-root transformed to remove skew and then normalised.

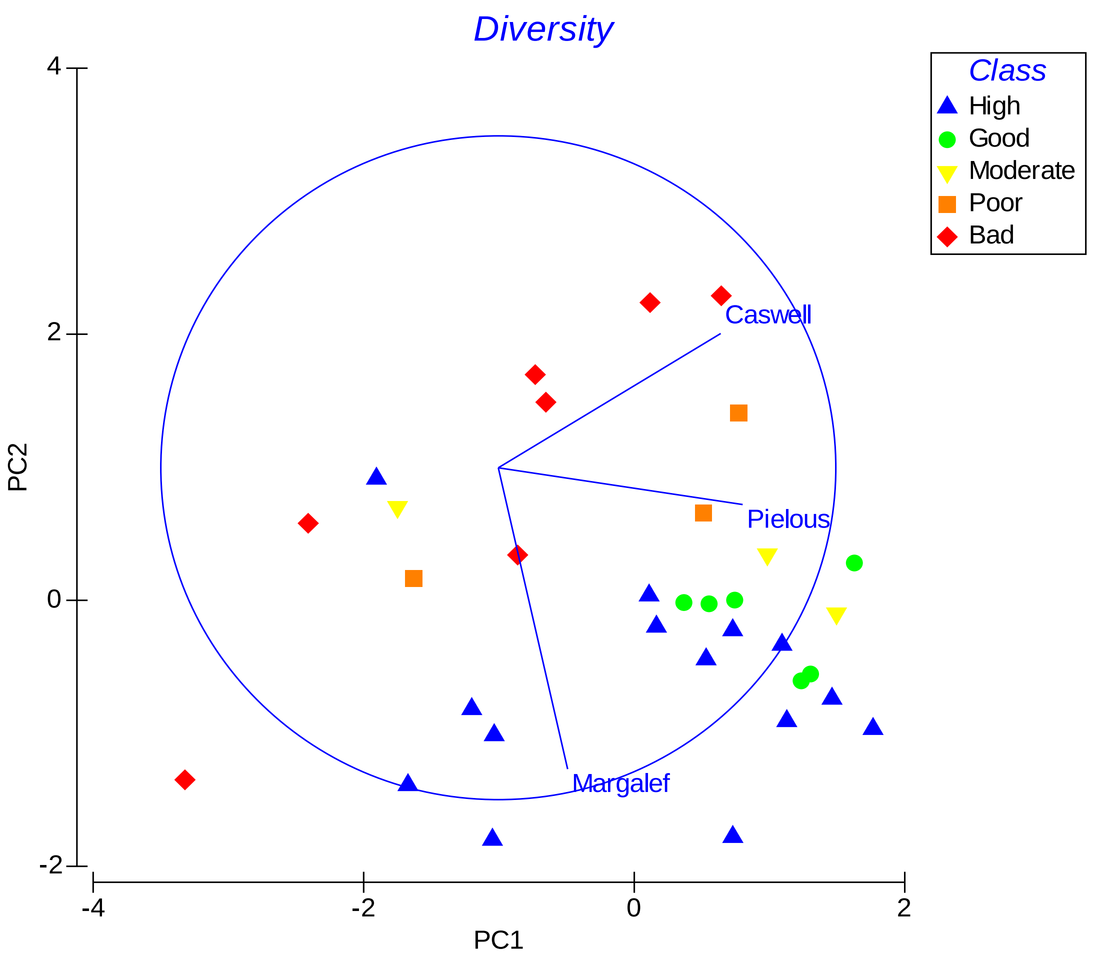
8.1.1 Diversity changes between class for Scottish humic sites

Figure 36 indicates there is some degree of separation between classes. Generally high and good status sites cluster towards the first axis. There appears to be no effect on diversity from high to good status. This might be expected with only minor disturbance. There appears to be a gradient of change from moderate to poor with bad status being more separated. Five out of the seven sites placed on the opposite side of the centroid to the high status sites, are classified as bad.

The high outlier is Carie Burn which is clearly different from many of the other high status sites because it has a low number of taxa (18) but much higher numbers of individuals (906). This in turn reduces evenness and lowers the metric scores. This pattern often typically represents an impacted community and so it is perhaps no surprise Carie Burn is closer to the bad status sites. Here, however, two of the species with high numbers in the sample are sensitive taxa (*Baetis rhodani* and *Rhithrogena semicolorata*). Addtionally, two other taxa are considered highly sensitive (*Glossosoma sp.* and *Perla bipunctata*). This does suggest that, although there may be other factors influencing the community at the Carie Burn site, WFD-AWICsp is robust enough to still classify this site as high in relation to acidification.

The bottom left-hand corner of the ordination has a bad status site (Benmeal) located closer to the high status sites, the nearest being Big Water of Fleet. These sites are clustered closer together because of their high number of taxa (>30) and uneven community structure. However, Big Water of Fleet has a number of highly sensitive and sensitive taxa, whereas Benmeal doesn't have any. Benmeal does have two moderately sensitive species (*Elmis aenea* and *Limnius volckmari*) and it must be questioned if a bad status site should contain any moderately sensitive taxa at all. This site though does have ANC values down to -17.1 µeq/l, suggesting a very acidified site and correct classification and perhaps that *E.aenea* and *L.volckmari* should be in the tolerant classes. More empirical data than were available would be required to determine this.

Figure 36: PCA analysis of Scottish humic sites (>10 mg/l DOC) using Caswell, Margalef and Pielou as environmental variables.



8.1.2 Diversity changes between class for Scottish clear-water sites

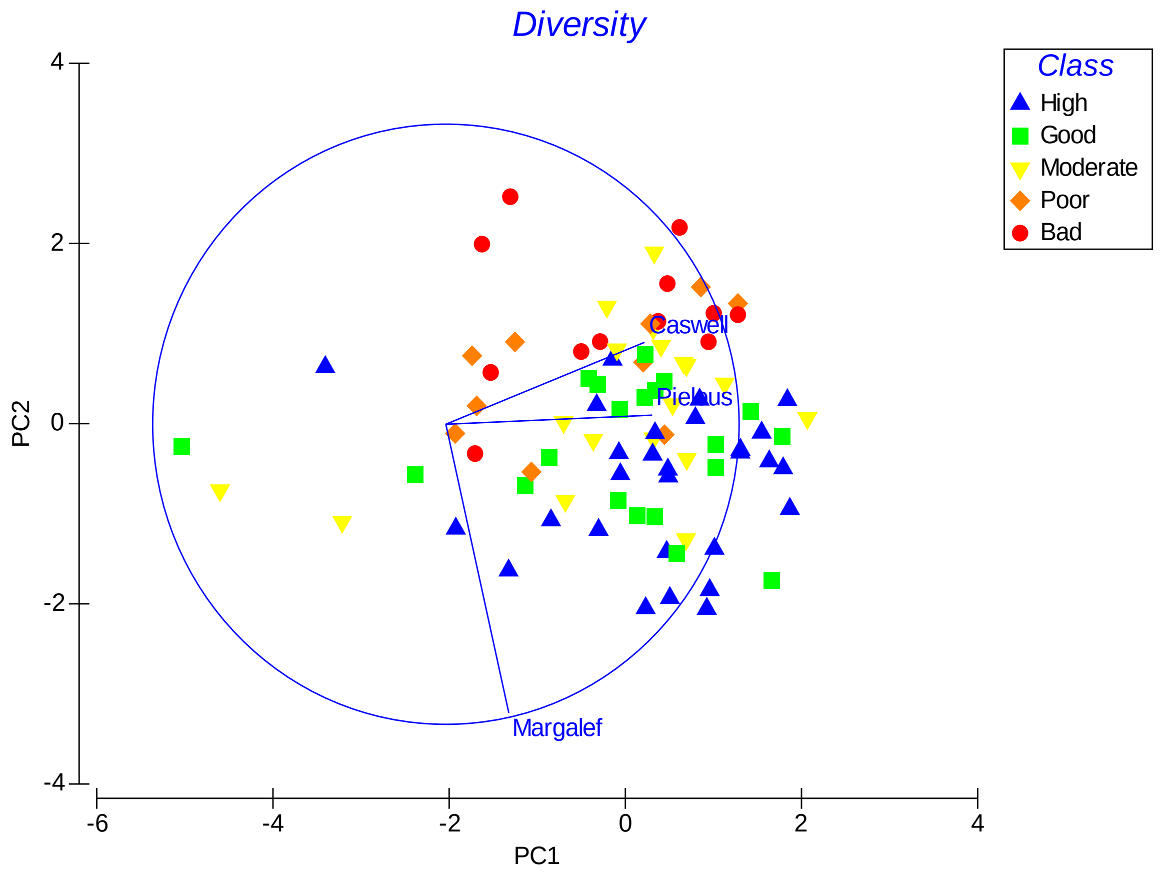
Figure 37 indicates there is little separation between classes. However, a general change in class can be seen from the first axis towards the top of the ordination. There appears to be little effect on diversity from high to good status. This might be expected with only minor disturbance. Sites in moderate status are highly variable in their level of diversity. This is indicated by the breakpoint in Shannon diversity occurring in the middle of moderate status and rapidly declining NTaxa (Figures 21 and 25). There appears to be more separation for poor and bad status classes, at least compared to most sites in good and high status.

The high status outlier toward axis 2 is the River Duror, which is clearly different from other high status sites because of its very low evenness and low NTaxa (14). All except two of the taxa are found in low numbers (<10). This might suggest an impacted site and an incorrect classification. However, one of the species found in high abundances is *Baetis rhodani* (with 343 individuals), a species considered sensitive. Additionally the sample also contains two other taxa that are considered highly sensitive (*Glossosoma sp.* and *Perla bipunctata*) as well as three more sensitive taxa (*Ecdyonurus sp, Rhithrogena sp.* and *Hydraena gracilis*). This does suggest that, although there may be other factors influencing the community at the River Duror site, WFD-AWICsp is robust enough to still classify this site as high for acidification.

The other high status site, clearly closer to poor and bad sites than to other high status sites, is the River Scaddle. Unlike the River Duror it is not the very low evenness which appears to cause this (most of the taxa have similar abundances) but the very low NTaxa (11), which is more typical of highly acidified sites. Again, although this might suggest mis-classification, the sample contains both highly sensitive (*Caenis sp*) and sensitive taxa (*Baetis rhodani, Ecdyonurus sp, Rhithrogena sp*). A number of good status sites also border bad and poor status sites in this area of the ordination. The two closest are NW5 and NW14 characterised by low NTaxa (11 and 13 respectively). However, in both cases the samples contain at least two sensitive taxa (NW5 has *B.rhodani* and *Rhithrogena sp* and NW14 has *B.rhodani, Ecdyonurus sp* and *Philopotamus montanus*) and at least two moderately sensitive taxa.

One of the poor status sites is positioned in among the high status sites. The site is Cardoon and has a surprisingly high number of taxa for a site classified as poor (17). Despite this, nearly all the taxa found are tolerant with only two species that are considered moderately sensitive (*E.aenea* and *L.volckmari*), both of which had low abundances. Additionally the chemistry supports the classification (ANC 9.35 µeq/l, pH 4.67). This does suggest that this site, although with a relatively diverse community, is acidified and therefore classified correctly.

Figure 37: PCA analysis of Scottish clear-water sites (<10 mg/l DOC) using Caswell, Margalef and Pielou as environmental variables.



8.1.3 Diversity changes between class for Welsh sites

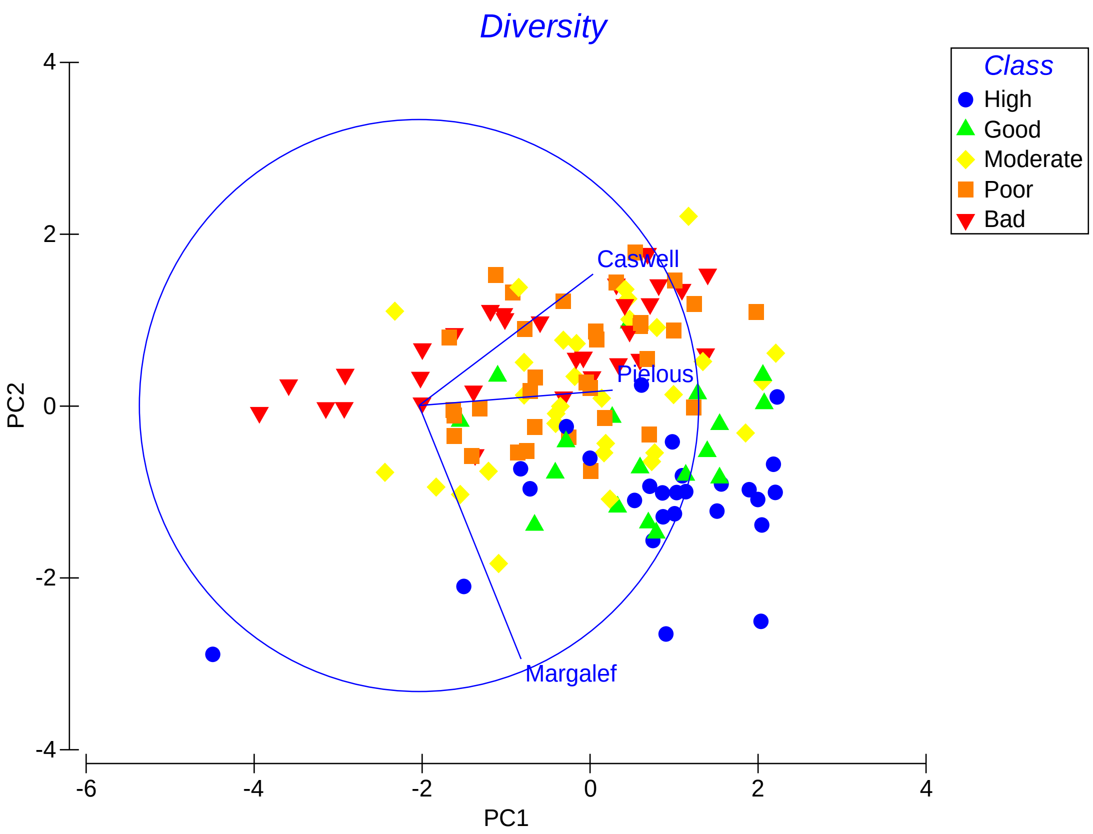
Figure 38 indicates that there is no separation between classes. However, much as for Scottish data, a general shift in class can be seen from the first axis towards the top of the ordination. There appears to be more of a difference in diversity from high to good status compared to Scottish clear-water sites. Like Scotland, sites in moderate status are highly variable in their level of diversity and this might suggest that this is the class where the biggest declines in diversity occur.

Generally sites at bad status are well separated from good and high status. There is a degree of overlap between poor status and high and good. Poor sites are more variable than for Scottish clear-water sites and this may suggest the Poor-Moderate boundary should be lower. The poor status site closest to the main cluster of high status sites (Cl1) is a relatively diverse site (e.g. NTaxa = 19). However, out of these taxa, only one is considered sensitive and the chemistry suggests the site is acidified with low ANC (2.37 µeq/l). This is supported by low pH (4.99), despite low DOC (0.8 mg/l).

Two sites in high status are placed very close to bad status sites. The Wye site, classified as high, is placed close to Nant Du, classified as bad. Both have low numbers of taxa (16 and 12 respectively) and uneven communities. However, the unevenness for the Wye is caused by large numbers of highly sensitive *Caenis sp.* wheras for Nant Du the uneven community is due to the tolerant stonefly, *Amphinemura sulcicollis.* The Wye site also contains a further three sensitive taxa wheras Nant Du contains none. Giedd, classified high, is placed close to Bryn Llyn Fawr. Again Giedd has low numbers of taxa for a high status site (15) but has two sensitive taxa (*Philopotamus montanus* and *Silo pallipes*) as well as four moderately sensitive species. Again, the bad status site has no sensitive taxa at all.

Two good status sites (I15 and Cothi) are placed close to the centre of the plot, surrounded by predominantly poor status sites. However, both these sites have a number of sensitive taxa (I15: *Baetis rhodani* and *Rhithrogena semicolorata*, Cothi: *Hydranea gracilis* plus four moderately sensitive taxa). In contrast, the poor site closest to Cothi (Llyn Brianne) has only one moderately sensitive taxon. The results indicate that the classification does represent a general change with respect to diversity, although a site can be poor in diversity but still have a number of sensitive taxa. These sites may be stressed from other pressures, suggesting that the classification is robust enough to still correctly classify high and good status for acidification.

Figure 38: PCA analysis of Welsh sites using Caswell, Margalef and Pielou as environmental variables.



8.2 Functional Groups

Functional group assessment investigates the relationship between status and functional groups, where feeding traits have been used as a proxy for ecosystem function. The assessment still includes grazers, shredders and parasites but also includes all other functional groups using AQEM (2004), to account for changes in function across the whole community. These are; predators, gatherers, miners, passive filter feeders, active filter feeders and xylophagus taxa. Principal Components Analysis (PCA) was used to assess diversity, with the diversity metrics treated as environmental variables. All metric scores were Log (X+1) transformed to remove skew and down-weight the influence of numerically dominant functional groups. The data were then normalised.

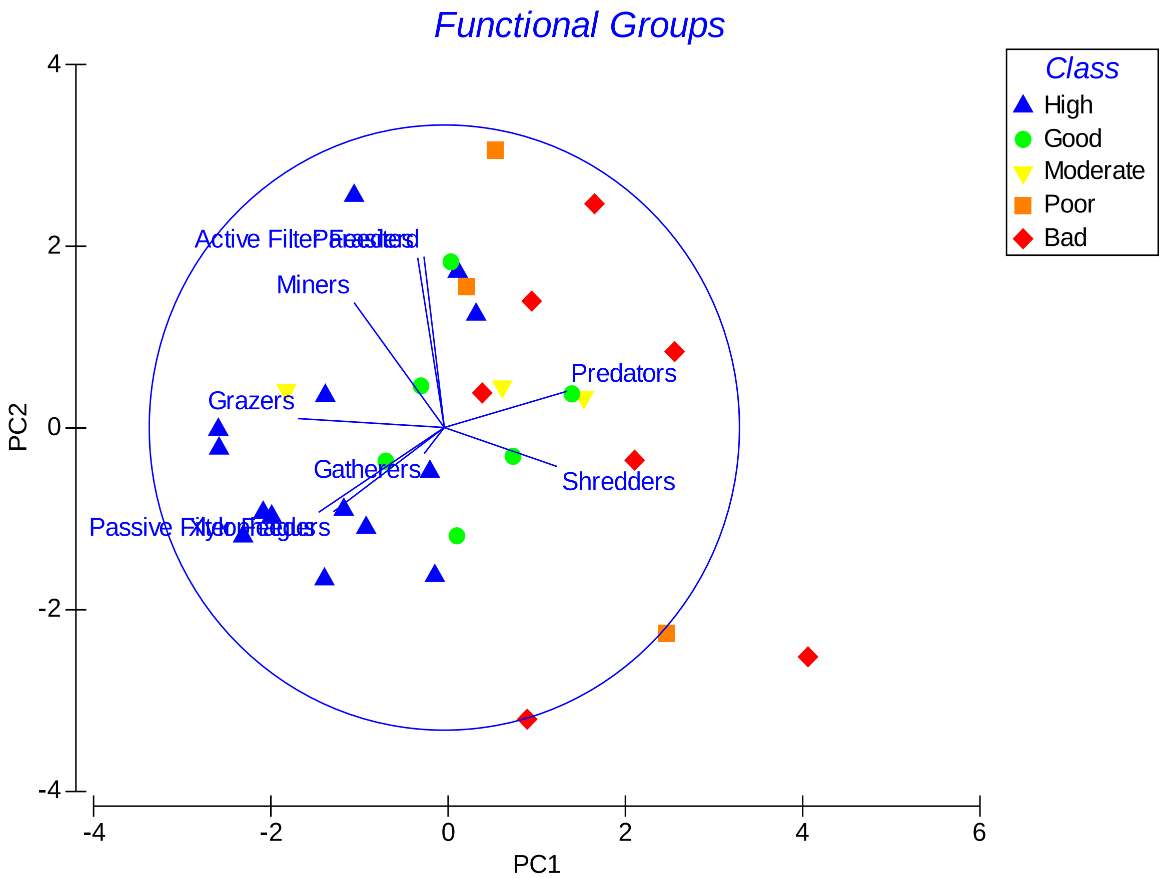
8.2.1 Functional changes between class for Scottish humic sites

There is a general gradient with high status sites clustering towards the left-hand side of the ordination plot, good and moderate status sites in the middle of the ordination (Figure 39). This provides evidence that the classification broadly reflects functional change although the scatter of good and moderate status sites implies large changes in function do not occur until significant levels of pressure are reached.

It is noticeable that sites in poor and bad status have very varied communities which is also evident from the diversity plot. This is surprising given that at highly stressed sites we might expect a more homogenous community. These highly stressed sites characteristically have low numbers of species and so the presence, or absence, of a couple of species between sites can make a significant difference to community structure. For example, the two bad status sites that are separated by the largest distance in the ordination (GA11, close to the first axis and GA28, towards the upper part of the ordination) have no sensitive taxa at all and both are dominated by tolerant stoneflies. However, GA11 has four species of Nemouridae, whereas GA28 has only two. Unlike many tolerant species, this family of stonefly has species which are grazers for at least part of their diet. In contrast, GA28 has three tolerant predators that are not found at GA11. Consequently, at bad status sites, with low taxa numbers, a few different species can make a large difference to their position within the ordination. Despite this, neither have any sensitive taxa, supporting the classification.

As predicted, shredders appear to be aligned towards the acidified sites and this is also the case for predators. Grazers are aligned towards the high status sites but not as clearly as xylophagus taxa and passive filter feeders (PFF). PFF may be sensitive to acidification due to lower levels of plankton at acidified sites. On a scatterplot of PFF v ANC, PFF increased with lowered ANC down to ANC 90 µeq/l, although the response of this group is unimodal. Below ANC 90 µeq/l this group declines to the most acidified sites. Xylophagus taxa demonstrated a noticeable unimodal response along the pressure gradient, peaking around 90-100 ANC µeq/l. Below ANC 80 µeq/l the group starts to decline. It is difficult to hypothesise why this response might occur. It may suggest taxa that depend on woody debris for at least some part of their life stages, are sensitive to acidification (e.g. some species of Elmidae), or that woody detritus becomes scarcer in acidified catchments. Although many acidified catchments have extensive standards of conifer plantations, wood from non-native conifers is generally sub-optimal for most xylophagus taxa. Commercial plantations often have trees similar in age, limiting the variation in size of woody debris entering the river system, and hence a reduction in the complexity of resource use among species of this functional group.

Figure 39: PCA analysis of Scottish humic sites (>10 mg/l DOC) using functional groups as environmental variables.

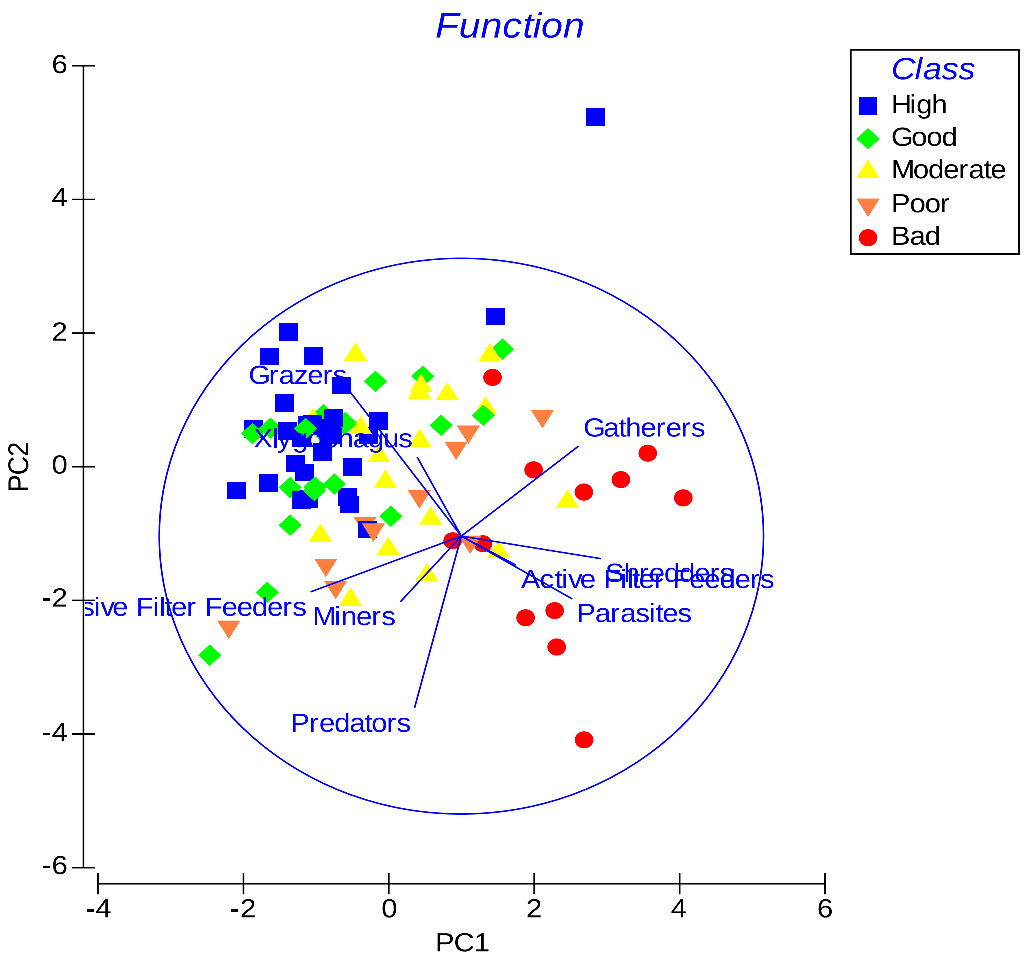


8.2.2 Functional changes between class for Scottish clear-water sites

There is a noticeable gradient with most high status sites clustering tightly towards the left-hand side of the ordination plot (Figure 40). Most good, moderate and poor status sites are also placed toward the left-hand side of the ordination plot but increasingly towards the centroid. This does provide evidence that the classification broadly reflects functional change. Despite this, only the bad status sites are well separated in the ordination suggesting that large scale functional change only occurs at very acidified sites. Bad status sites are well separated from each other. This is surprising given that at highly stressed sites we might expect a more homogenous community. These highly stressed sites do characteristically have low numbers of species and so the presence, or absence, of a couple of species between sites can make a significant difference in community structure. One of the bad status sites (NW20) is placed among moderate and good status sites towards the upper part of the ordination. This site has high numbers of grazers for a site classified in bad status (34%) and low numbers of parasites (0.5%). Despite this, the site does not have any sensitive taxa, with a very typical fauna for an acidified site, supporting the classification.

As predicted, and in common with humic sites, shredders appear to be aligned towards the acidified sites. Contrasting to the humic sites, parasites also appear aligned to acidified sites agreeing with the original prediction that parasitic loading increases with increased stress. Predators are less clearly aligned to acid stressed sites compared to humic sites. Grazers and xylophagus taxa are again aligned towards the high status sites but this time passive filter feeders are not.

Figure 40: PCA analysis of Scottish clear-water sites (<10 mg/l DOC) using functional groups as environmental variables.



8.2.3 Functional changes between class for Welsh sites

There is a very clear split with nearly all the good and high status sites clustering on the right hand side of the plot (Figure 41). They are tightly clustered suggesting sites with minimal acidification pressure are functionally fairly similar. This was also the case for Scottish clear-water sites. There is a very clear split of both poor and bad from good and high with moderate status approximately evenly split between the two sides of the ordination. This is in contrast to Scotland where most good, moderate and poor status sites clustered close to high and good and only bad status sites appeared to be functionally different. This may suggest that, because significant functional change only occurs in Scotland at bad status, compared to poor or moderate status in Wales, that the boundaries are in different places along the pressure-impact gradient. However, we might expect any step-change in function to occur in moderate status and this supports the position of the Welsh boundaries.

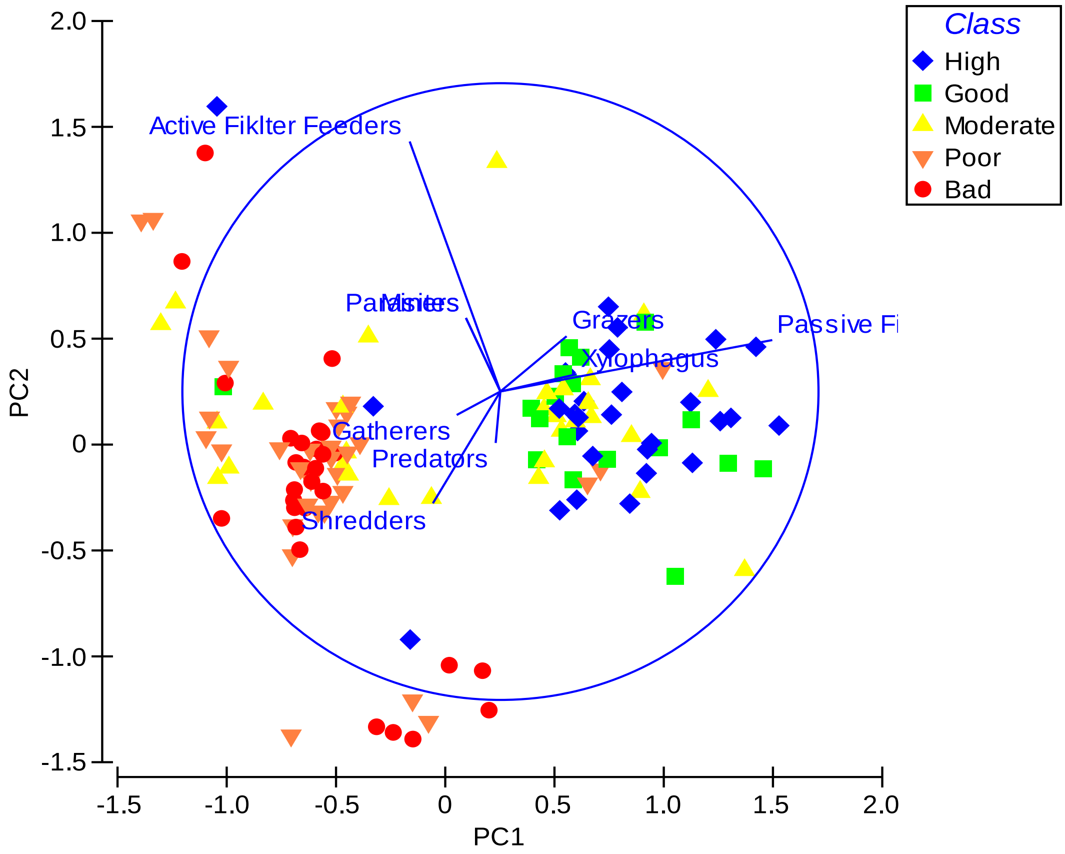
Three high status sites are placed on the left-hand side of the ordination. The site towards the first axis (CW11) has been placed with a cluster of bad and poor sites characterised by high percentages of gatherers with low numbers of passive filter feeders, xylophagus taxa and miners. Low proportions of passive filter feeders especially, seem typical of acidified sites and this might suggest this site has been incorrectly classified. CW11, however, has two highly sensitive species (*Alainites muticus* and *Gammarus pulex*) and four more sensitive taxa. Therefore, although this site might be functionally atypical for high status sites, there is no evidence it is acidified. The Lledr is placed in the top left-hand corner of the ordination along way from other high status sites. Not surprisingly this is an unusual site in terms of its functional groups. It has no passive filter feeders but has a very high proportion of active filter feeders (9.2% compared to a mean for all sites of 1.8%). However, it also has a low percentage of predators and a very high proportion of grazers (52.3% compared to the mean of 28%), more typical of non-acidified sites.

The third atypical high status site (Caletwr) is placed closer towards the middle of the ordination and appears to be placed here because of the low proportion of passive filter feeders (0.7% compared to a mean of 2.6%). Although low percentages of passive filter feeders seem typical for acidified sites, Caletwr also has high proportions of grazers (42.1%) but low shredders (9.3%, mean of 14.1%), more typical of high status sites. However, Caletwr has an EQR right on the boundary of high-good (0.981) and so there must be high uncertainty whether this site really is at high status. The poor diversity (Caletwr is one of the high sites placed away from the main high status cluster in Figure 39) means the presence of only one sensitive taxon (*Baetis rhodani*) and two moderately sensitive taxa, has elevated the EQR above 0.98. Therefore, there must be considerable doubt that this site has been classified correctly.

One good status site is placed close to the second axis (II5) because of a high proportion of parasites (1.1% compared to the mean of 0.6%) and having no passive filter feeders. The presence of three sensitive mayflies suggest that this site is correctly classified. Three poor sites are placed on the right-hand side of the ordination within the mainly good and high cluster. The three sites (LI4, CI1 and Preseli 3) are placed to the furthest right mainly because of their high percentage of passive filter feeders, more typical of good and high status. All three communities are dominated by tolerant taxa, hence the low EQR. However, each of the three samples contain one sensitive taxon, the same for each site (*Wormaldia sp*). This might suggest moderate status would be more realistic for these sites, although it might also imply that *Wormaldia sp* are rather more tolerant than their assigned sensitivity class. Preseli 3 has a very low ANC (-19.7µeq/l) and CI1 also has low ANC (2.37µeq/l), supporting this argument. The chemistry, therefore, supports the classification for Preseli 3 and CI1. LI4 has a higher ANC (28.2 µeq/l) and so there must be more doubt over its status.

As predicted, and in common with Scottish sites, shredders appear to be aligned towards the acidified sites. In contrast to the Scottish clear-water sites, parasites are not clearly aligned to stressed sites and predators are less clearly aligned to acid stressed sites compared to the Scottish humic sites. Grazers and xylophagus taxa are again aligned towards the high status sites in agreement with both Scottish types. Passive filter feeders are also strongly aligned to high and good status.

Figure 41: PCA analysis of Welsh sites using functional groups as environmental variables.



8.3 Community Structure

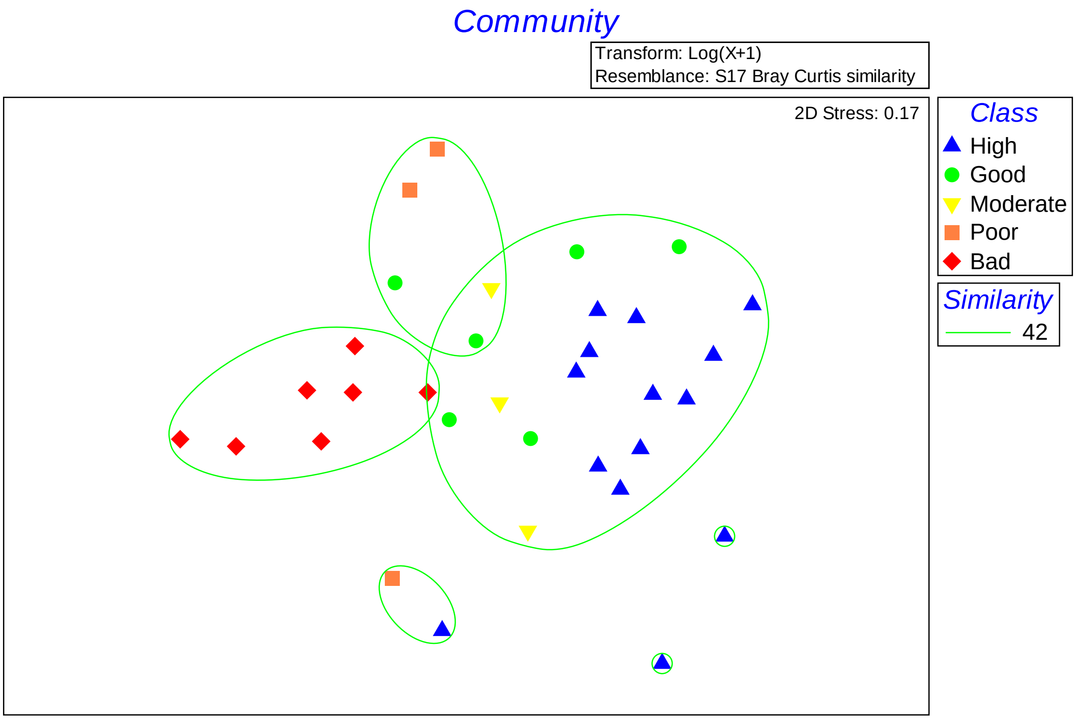
The community assessment includes all taxa found in the samples. Again, this assessment is not totally independent from the boundary setting and a certain amount of caution needs to be applied here. The analyses looks at the raw structural differences between sites and WFD-AWICsp is a structural metric, consequently there is a certain amount of circularity. Despite this, WFD-AWICsp only looks at a component of the community (i.e. there are only 48 taxa in the metric) and so there is still value in looking at the community as a whole.

Data were Log (x+1) transformed, after removing rare taxa (found in less than 3% of samples), to down-weight contributions of quantitatively dominant groups or taxa. This transformation was preferred to less heavy methods (e.g. square-root) due to the presence of very high abundances of taxon groups in some samples (e.g. Chironomidae). The transformed data were then fed into a similarity matrix using the Bray-Curtis coefficient. Bray-Curtis examines differences between all pair-wise observations where S=0 if the two samples have no species in common and S=100 if the two samples are identical. Bray-Curtis has the advantage over many other coefficients because the value is unchanged by the inclusion of joint absence. Non-metric Multidimensional Scaling (MDS) was then carried out on the similarity matrix (50 re-starts, Kruskal fit) where all taxa are treated as variables. Because this analysis uses biological data MDS was preferred over PCA. MDS constructs a sample 'map' whose inter-point distances have the same rank-order (hence non-metric) as the corresponding dissimilarities between the samples (i.e. the Bray-Curtis matrix). The CLUSTER program was run on the Bray-Curtis matrix using the group-average link and the SIMPROF function overlaid on the resulting dendrogram. In this case SIMPROF was run on 1000 permutations looking for significant clusters (p = <0.05) in samples which were not *a* *priori* divided. Slices across the dendrogram, where all clusters were significant, are shown on the MDS plots as contours.

8.3.1 Community changes between class for Scottish humic sites

There is a clear gradient, with high status sites clustering towards the right-hand side of the ordination plot (Figure 42). The stress is relatively high (0.17) and so, although giving a useful 2-dimensional picture of the ordination space, the results of the CLUSTER analysis are overlaid as contours to aid interpretation. This suggests for most of the high, good and moderate status sites, there is no significant difference in community (where SIMPROF $p = 0.05$). The high status sites do, however, remain separate in the ordination space. Additionally, all bad status sites cluster into the same group and this adds support to the location of the poor-bad boundary. Carie Burn (high status) and Rogie Burn (poor status) are placed in the same cluster and are close in the ordination space. Whilst the two sites do generally have similar communities there are differences between the two. Rogie Burn only has one moderately sensitive species (*E.aenea*) whereas Carie Burn has two highly sensitive taxa, two sensitive taxa and three moderately sensitive taxa. The cluster to the top of the ordination contains two poor status sites and two sites classified as good status. Whilst the communities are reasonably similar, again there are differences between them. The poor status sites, GA19 and GA17 only contain one and two moderately sensitive taxa respectively. In contrast the good status sites, GA18 and NW22, contain a number of sensitive taxa (three MS, one S and one MS, two S, one HS respectively).

Figure 42: non-metric Multidimensional Scaling (MDS) plot derived from a Bray-Curtis similarity matrix of Scottish humic sites (>10 mg/l DOC)



8.3.2 Community changes between class for Scottish clear-water sites

Figure 44 investigates the relationship between status and the invertebrate community using a CLUSTER analysis. The stress for the MDS was >0.2 (Figure 44) and so the results from the CLUSTER analysis are shown, often giving a better overall representation of the relative differences between sites.

The MDS clearly represents a general gradient of pressure change from left to right, if class can be considered a proxy of acidification (Figure 43). Generally the classes high, poor and bad are positioned as expected. There is no mixing between high and poor/bad within the ordination. Most moderate sites are also placed as expected, although there are some outliers and this must account for much of the high stress in the 2-D plot. For example, the bottom moderate site is actually closely related to the moderate status site at the top of the plot (they are placed in the same cluster in Figure 44). Sites classified as good are the most scattered, possibly suggesting that this is the class where we are most uncertain in status. Four good status sites are placed very close to the grouping of poor status within the ordination. These are; NW10 and NW14, which are close together, and NW13 and GA27. The two former sites are closely related to Bealach East, which is classified as poor. Although overall the sites are very similar, Bealach East only has two moderately sensitive taxa, both in low abundance. In contrast NW13 has three sensitive and three moderately sensitive taxa, NW10 has one highly sensitive, two sensitive and three moderately sensitive taxa. The similarity between the three sites is driven by a large number of tolerant taxa (including taxa suspected of being tolerant but not included in WFD-AWICsp e.g. *Leuctra sp* and *Polycentropus flavomaculatus*). These findings are also similar for NW13 and GA27, both of which have a number of sensitive species compared to the closest site in poor status (Carrick Lane), which has none. This suggests that the classification is robust enough to account for community similarity and still identify crucial differences between sites.

In the CLUSTER analysis (Figure 44), generally the high and good status sites cluster into the same groups. This suggests there is no difference in the community as a whole between these two status classes. No high status sites appear in the same cluster as any bad status sites although one site (Allt Gheallaidh) is in the same cluster as a poor status site (Bealach East). However, unlike Bealach East, which has no sensitive taxa, Allt Gheallaidh has four. The good status site Abhainn Ruadh also appears in this cluster but has two sensitive taxa. Four other good status sites are in clusters containing poor and bad sites but in each case the presence of sensitive taxa differentiates them, despite having similar communities. Overall, 96% of high status sites and 75% of good status sites are within what could be termed 'sensitive' clusters. Out of the poor and bad status sites, 100% are within what could be termed 'tolerant' clusters. This might suggest we can be more confident in our classification of poor and bad status sites compared to high and good. Sites at moderate status are more scattered across the classes, perhaps as expected, although 72% are within 'tolerant' clusters. It is clear from the level of similarity that many of the 'sensitive' clusters have closer similarity to 'tolerant' clusters than to other clusters with mainly high and good status sites. This implies there are many other factors driving community differences in these data, for example, regional differences in taxon distribution. The classification appears robust enough to account for these.

Figure 43: non-metric Multidimensional Scaling (MDS) plot derived from a Bray-Curtis similarity matrix of Scottish clear-water sites (<10 mg/l DOC)

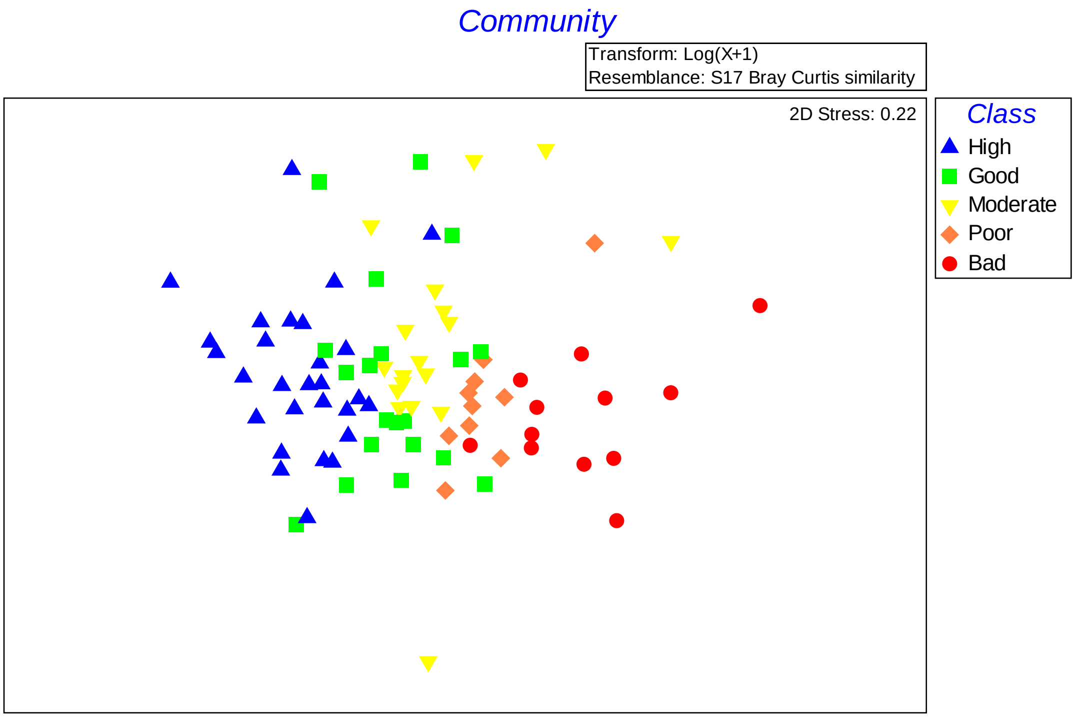
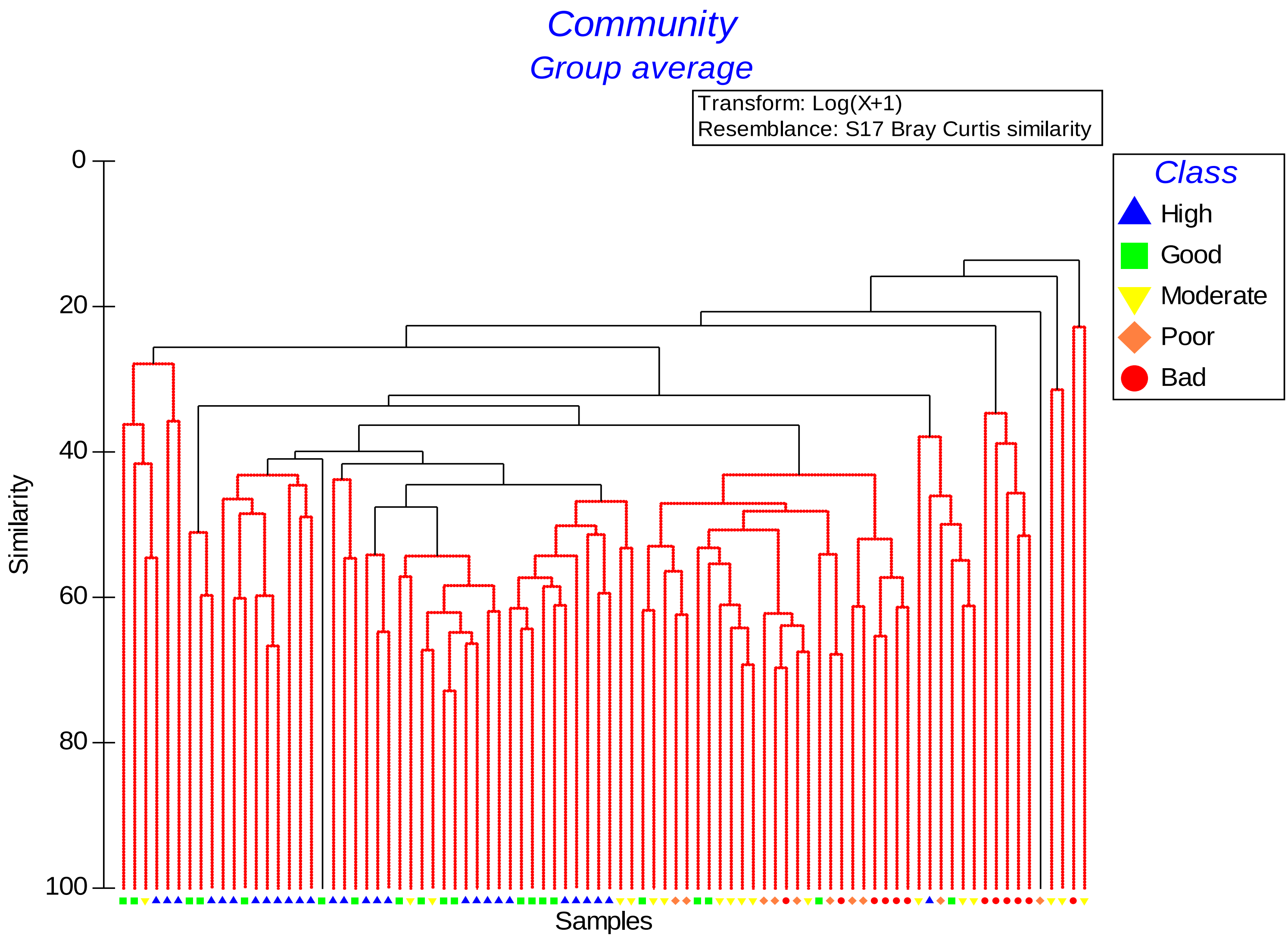


Figure 44: Dendrogram of CLUSTER analysis derived from a Bray-Curtis similarity matrix of Scottish clear-water sites (<10 mg/l DOC). Solid black lines represent significant clusters using SIMPROF (where significance $p = 0.05$)



8.3.3 Community changes between class for Welsh sites

Figure 46 investigates the relationship between status and the invertebrate community using a CLUSTER analysis. The stress for MDS is >0.2 (Figure 45) and so the result from the CLUSTER analyses is shown, often giving a better overall representation of the relative differences between sites.

The MDS clearly represents a general gradient of pressure change from right to left, if class can be considered a proxy of acidification (Figure 45). Generally the classes high, poor and bad are positioned as expected. With the exception of one high status site, there is no mixing between high and poor/bad within the ordination. Most intermixing of class are for moderate and good status. This may suggest where we are most uncertain about classification. There are two high status outliers; Giedd and Caletwr. Giedd was also an outlier in the diversity plot with low numbers of taxa for a high status sites (15) but it does have two sensitive species (*Philopotamus montanus* and *Silo pallipes*) and several moderately sensitive taxa. Caletwr was identified as an outlier in the function plot and, to a lesser extent, the diversity plot. Given the only sensitive taxa present is *Baetis rhodani*, there must be some doubt that this site is in high status. There is one poor status site (SN733833) that is placed some way towards the high status sites, although it is surrounded by predominantly moderate status sites. Indeed, the site has an EQR that is very close to the moderate boundary (0.775). Its position within the ordination might suggest that it would be better classified as moderate and given the EQR. This perhaps suggests that the Moderate-Poor boundary should be slightly lower. The site has a diverse fauna more typical of less acidified sites and, although dominated by tolerant taxa, it does have one sensitive species (*Hydraena gracilis*) and two moderately sensitive species (*Elmis aenea* and *Limnius volckmari*). Despite this, the site has a very low ANC (-2.54 µeq/l) and therefore very likely to be acidified. This provides support that the classification is correct but does call into question as to whether *Hydraena gracilis* should be considered sensitive.

In the CLUSTER analysis (Figure 46), generally the high and good status sites cluster into different groups. This contrasts to Scottish sites and suggests there are differences in the community as a whole between these two status classes. No high status sites appear in the same cluster as any bad status sites although one site (Caletwr) is in the same cluster as a poor status site (Llwyd). Overall, 96% of high status sites and 67% of good status sites are within what could be termed 'sensitive' clusters. Therefore, 33% of good status sites are within 'tolerant' clusters suggesting a relatively high degree of uncertainty at good status. This may suggest that the Good-Moderate boundary should be higher, reducing the number of good status sites within the 'tolerant' clusters. However, moderate status already dominates the contour plot (Figure 36). Raising the EQR boundary would increase this dominance and this seems counterintuitive. Additionally, there is no evidence from Figure 28 to suggest the boundary should be higher. Out of the poor and bad status sites, 100% are within what could be termed 'tolerant' clusters. This suggests we can be more confident in our classification of poor and bad status sites compared to high and good. Sites at moderate status are more scattered across the clusters, perhaps as expected, although most are within 'tolerant' clusters (83%). Some of the clusters containing predominantly high and good sites have closer similarity to 'tolerant' clusters than to other clusters with mainly high and good status sites, although this pattern is less clear than for Scottish clear-water sites. This suggests there are other factors driving community differences in these data, for example, regional differences in taxa distributions. The classification appears robust enough to account for these.

Figure 45: Non-metric Multidimensional Scaling (MDS) plot derived from a Bray-Curtis similarity matrix of Welsh sites.

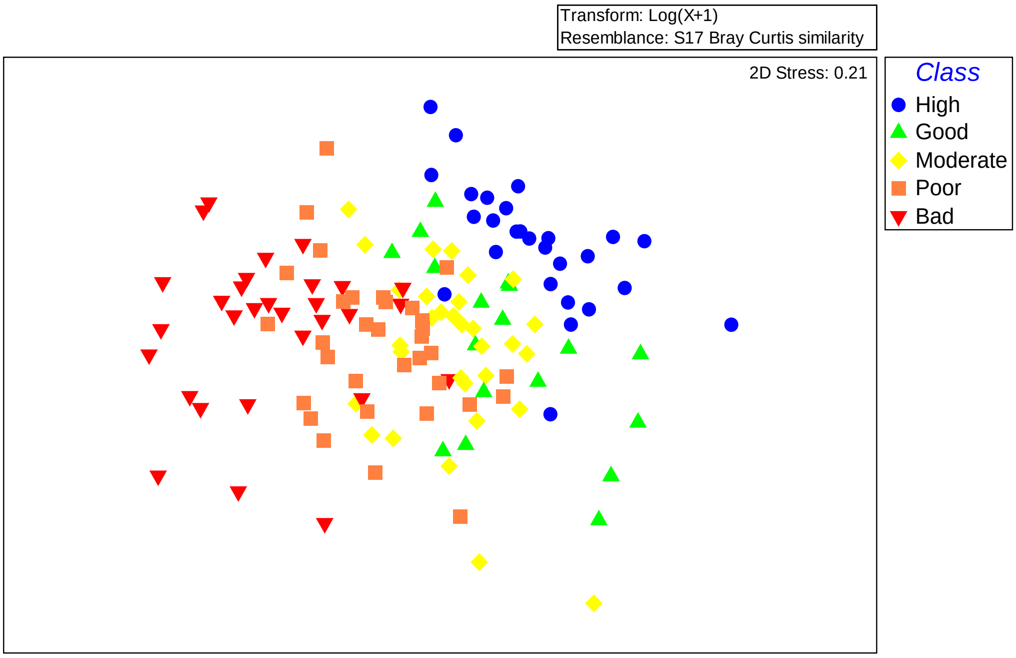
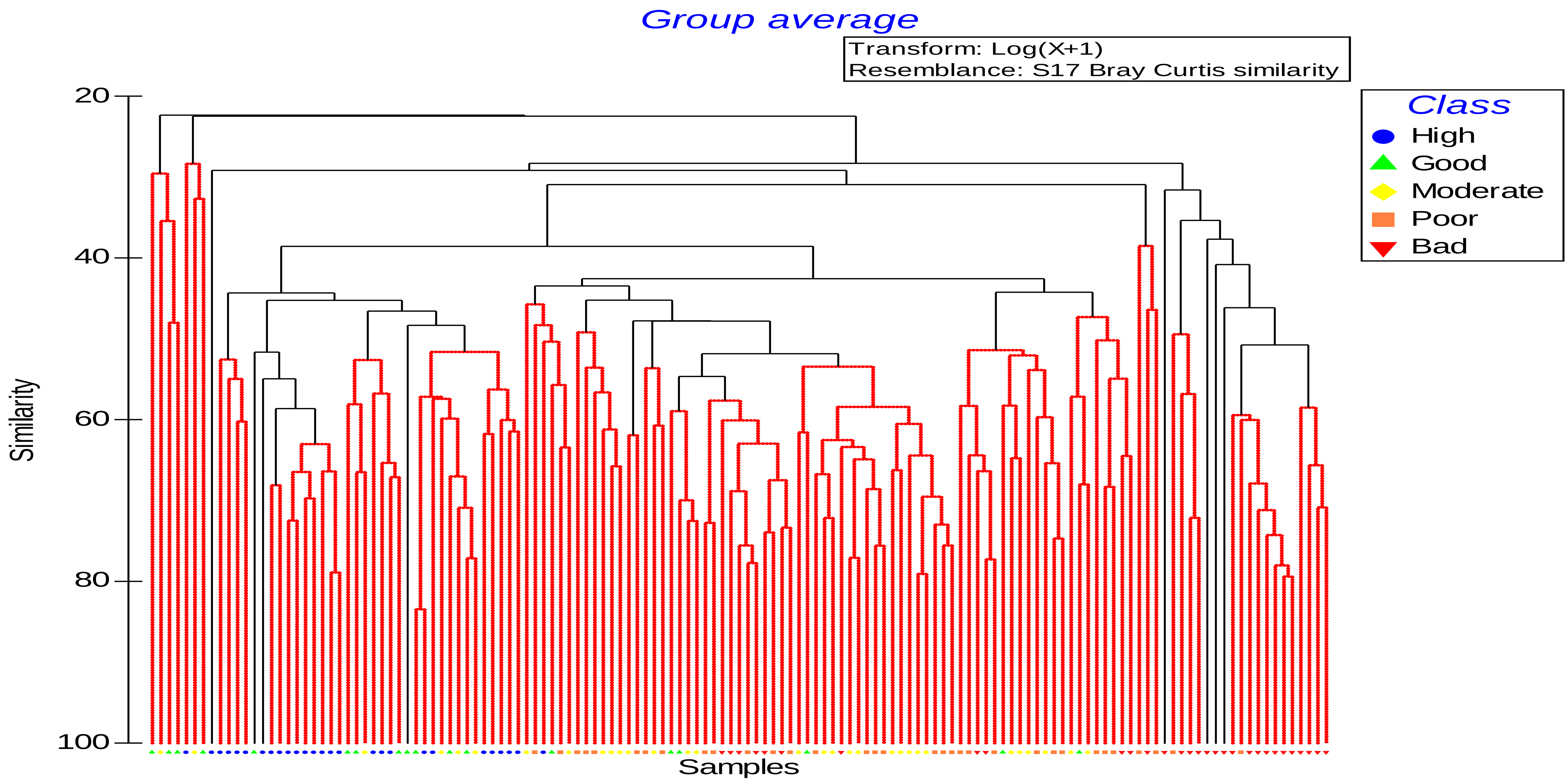

Transform: Log(X+1)
Resemblance: S17 Bray Curtis similarity
2D Stress: 0.21
Class
High
Good
Moderate
Poor
Bad

Figure 46: Dendrogram of CLUSTER analysis derived from a Bray-Curtis similarity matrix of Welsh sites. Solid black lines represent significant clusters using SIMPROF (where significance $p = 0.05$)


Group average
Transform: Log(X+1)
Resemblance: S17 Bray Curtis similarity
Class
High
Good
Moderate
Poor
Bad
Similarity
20
40
60
80
100
Samples

9.0 Final class boundaries

The final boundaries can be seen in Table 21 for each of the three types with corresponding WFD-AWICsp, ANC and pH values.

Table 21: EQR boundaries with corresponding ANC and pH values for each typology

	Scottish humic-water type				**Scottish clear-water type**				**Welsh type**			
	EQR	**WFD-AWICsp**	**ANC**	**pH**	**EQR**	**WFD-AWICsp**	**ANC**	**pH**	**EQR**	**WFD-AWICsp**	**ANC**	**pH**
High-Good	0.94	6.834	80	5.1	0.91	7.836	80	6.06	0.98	7.498	75	6.45
Good-Moderate	0.84	6.107	40	4.55	0.83	7.147	50	5.58	0.88	6.733	40	5.95
Moderate-Poor	0.78	5.671	15	4.22	0.72	6.2	10	4.9	0.78	5.968	5	5.44
Poor-Bad	0.74	5.38	0	4.03	0.66	5.683	-10	4.55	0.67	5.126	-30	4.89

Some of the boundaries are more uncertain than others and are discussed below.

9.1 Questionable Boundaries

Humic Scottish Poor-Bad boundary – Lack of data makes this boundary more uncertain. Using the LAMM method for boundary development (McFarland *et al*, 2009) results in a lower boundary EQR (0.67) and 0.73 appears to be rather high. Figure 21 predicted bad status up to ANC 50 µeq/l if the pH is <4.4. It seems unlikely that a humic site would be at bad status with such a high ANC, although it is rare a site would have such a high ANC, with such a low pH, even in humic waters. Furthermore, the multivariate analysis does suggest the bad status sites are well separated from other classes, especially when looking at the whole community.

Clear Scottish Poor-Bad boundary – Fewer data are present at this point in the pressure gradient. Boundary setting is also made more difficult by the linear response of all the response metrics, although the multivariate analysis does indicate a reasonable degree of separation between poor and bad sites. Using the contour plot, bad status is not predicted until approx ANC 0 µeq/l and pH 4.65, which seems reasonable.

Welsh High-Good boundary – In contrast to Scottish humic sites, WFD-AWICsp declines through the high status band. For Scottish clear-water sites, whilst WFD-AWICsp also declines, the slope of decline is not as steep. Additionally, there is no evidence of declining diversity or functional change. At Welsh sites most pressure metrics decline through the high status band and this implies the boundary needs to be higher. However, there is no evidence of changes in percentages of grazers and shredders, although parasites do increase. Furthermore, whilst passive filter feeders decline through high status, there is a distinct breakpoint at ANC 75 µeq/l. The relatively high pH at the high-good boundary (6.45) suggests the boundary should not be higher. This value is higher than the Scottish clear-water typology (6.06) and may reflect the lower buffering due to lower humic acids. The high EQR value for the High-Good boundary implies that the reference value may be too low, given most of the metrics decline through the high status band. The high EQR helps to compensate for this. The degree of separation of high and good sites in the multivariate analysis is actually greater than for Scottish sites and this helps support this boundary for Wales.

Welsh Moderate-Poor boundary – Poor status sites in the PCA of diversity and community are scattered across much of the plot. This might suggest the boundary should be lower. Whilst the ANC is at a level that could be expected at this boundary (5 µeq/l), the pH might be considered rather high (5.44). The PCA plot of functional groups shows a clear split of poor and bad sites from high and good but moderate is split between both sides of the ordination. Lowering the EQR would actually change more of the sites on the left-hand side of the plot to moderate.

9.2 Questionable Species

Some species appear to be potentially placed in an incorrect sensitivity class. It is not proposed for these species scores to be changed, as more empirical data would be required. However, in any classification, where there is a high degree of uncertainty (e.g. a site is placed on, or very close to, the Good-Moderate boundary), the presence of these taxa should be considered.

Elmis aenea and *Limnius volckmari* – Both species were identified as being more tolerant than their original highly sensitive class by Rendall *et al* (2009) and McFarland (2009). The final AWICsp (Murphy, 2009) also suggested the original class was incorrect, reclassifying both species as moderately sensitive. However, both taxa are found at bad status sites. It is questionable whether any moderately sensitive taxa, even at low abundances, should be present at sites in bad status. Generally, the low pH and ANC values at these sites, coupled with the absence of all other moderately sensitive taxa, supports the classification. Despite this, there are no obvious cases where the presence of these two species result in an apparently incorrect classification.

Wormaldia sp. – This genus is found at a number of poor status sites, some of which have very low ANC. The sensitivity class was questioned by McFarland (2009) and therefore there must be some uncertainty over its true class. It is possible that some species of *Wormaldia* are more tolerant than others.

Hydraena gracilis – This sensitive species is also found at sites in poor status with correspondingly very low ANC (down to -2.54 µeq/l), although only in two cases. This species was not raised in either the Rendall *et al* (2009) or McFarland (2009) reports.

10.0 Summary and future work

Reference sites were chosen a using chemical and biological screening. Significant differences in reference communities were found between Welsh and Scottish sites. Naturally humic reference sites in Scotland were also found to have distinct macroinvertebrate fauna meriting a separate typology. Taxa accounting for within-type community similarity were chosen to derive expected WFD-AWICsp values.

Boundaries were based on structural and functional metrics and their changes along the pressure gradient. These were validated using multivariate analysis looking at different aspects of the whole community.

WFD-AWICsp enables rivers throughout the UK to be classified for acidification. Models have been developed specifically for Scottish and Welsh rivers. However, English sites can be compared to the expected community for Wales and compared to the Welsh boundaries.

It is recommended chemical sampling is taken on a monthly basis to increase the chance of capturing episodic acid events and hence support the classification. The relatively high Acid Neutralising Capacity (ANC) values at which community change was noted suggest many of these events have been missed. Chemical data that are required to support the classification are Cantrell ANC (Cantrell, 1990), pH and Dissolved Organic Carbon (DOC). The inclusion of inorganic monomeric aluminium will improve ANC measurements.

Whilst it is apparent this method can produce a reliable classification for WFD, it is recommended that ultimately WFD-AWICsp is embedded within RICT. To do this successfully RICT will need to incorporate predictor variables that are independent from pressure. These should include modelled alkalinity and DOC. Additionally, some consideration over the number of reference sites from rivers at risk from acidification included in the RIVPACS dataset needs to be carried out. A larger number of more suitable reference data may be required. This is critical for a more accurate site-specific predicted fauna. It is recommended the boundaries remain unchanged once WFD-AWICsp is embedded within RICT.

References

AQEM consortium (2004) AQEM European stream assessment program. Version 2.3.

Caswell H (1976) Community structure: a neutral model analysis. *Ecol Monogr*. 46:327-354

Cantrell KJ, Serkiz SM, Perdue EM. 1990. Evaluation of acid-neutralising capacity data for solutions containing natural organic acids. *Geochim Cosmochim Acta* 54: 1247-1254

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

Davy-Bowker J, Murphy JF, Rutt GP, Steel JEC, Furse MT. 2005. The development and testing of a macroinvertebrate biotic index for detecting the impact of acidity on streams. *Archive für Hydrobiologie* 163: 383-403.

Extence CA, Balbi DA, Chadd RP. 1999. River flow indexing using British benthic macroinvertebrates: A framework for setting hydroecological objectives. *Regulated Rivers: Research & Management* 15: 543-574

Kelly M (2005) Nutrient and acid conditions required to support good ecological status in benthic diatom assemblages in rivers. RTT Report 56.

Ledger ME, Hildrew AG (2005) The ecology of acidifcation and recovery: changes in herbivore-algal food web linkages across a stream pH gradient. *Environmental Pollution* 137: 103-118.

Lafferty KD and Kuris AM (1999) How Environmental Stress Affects the Impacts of Parasites. *Limnology and Oceanography* 44: 925-931

McFarland B, Carse F, Sandin L (2009) Littoral macroinvertebrates as indicators of lake acidification. *Aquatic Conservation*. Currently in early view.

McFarland B (2009) The development of a Water Framework Directive compliant method for the assessment of acidification using macroinvertebrates – Re-appraisal of AWICAR5log species sensitivity classes. Unpublished report to Rivers Task Team.

Moe SJ, Schartau AK, Baekken T, McFarland B (2009) Assessment of macroinvertebrate metrics for classification of acidified rivers: analysis of monitoring data from UK, Norway and Sweden. *Freshwater Biology* in press

Monteith DT, Simpson GL (2007) Macroinvertebrate classification diagnostic tool development. Project WFD60. SNIFFER, Edinburgh.

Murphy J (2009) Revision of AWICsp biotic index. Unpublished report to Rivers Task Team.

Ormerod SJ, Durance I (2009) Restoration and recovery from acidification in upland Welsh streams over 25 years. Journal of Applied Ecology. 46: 164-174

Petrin Z, Laudon H, Malmqvist B (2007) Does freshwater macroinvertebrate diversity along a pH-gradient reflect adaptation to low pH? *Freshwater Biology* 52: 2172-2183

Petrin Z, Laudon H, Malmqvist B (2009) Diverging effect of anthropogenic acidification and natural acidity on community structure in Swedish streams. *Science of the Total Environment* 394: 321-330.

Rendall D, Bell A, Doughty R, Cook M (2009) DF09-07 A Preliminary Review of the AWICspAR5Log Method for WFD Assessment of Acidification in Scottish Rivers. Unpublished report to Rivers Task Team.

Wilkinson DM (1999) The disturbing history of intermediate disturbance. *Oikos* 84: 145-147