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The relationship between epilithic diatom assemblages and water chemistry in Scottish streams

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### 1 Introduction

During the late 1970s and early 1980s it became apparent that a large number of lochs and streams draining base-poor catchments in various parts of Scotland had been acidified, and had impoverished fish and invertebrate communities. The Scottish Acid Waters Baseline Study was therefore initiated in 1986 with the aim of surveying the current distribution and extent of acid rivers and streams in Scotland, and to provide a baseline of chemical and biological data against which future changes may be assessed.

The survey involved a three-year sampling program of stream-water chemistry, benthic invertebrates and epilithic diatoms. This report presents results from the diatom component of this study, and detailed description of the chemistry and diatom data from the survey sites.

In addition to documenting the diatom dataset, this interim report also examines the relationship between the diatom assemblages and environmental factors, and develops a predictive model for the bio-monitoring of stream-water pH.

I should like to thank members of the Scottish RPBs for collecting diatom samples and Ross Doughty of the Clyde River Purification Board for collating and transferring water chemistry data. The project was funded by a Department of the Environment grant to Prof. R.W. Battarbee.

### 2 Methods

### 2.1 Site Selection

Diatom and water samples were collected from 149 sites distributed among the seven Scottish River Purification Boards according to the size of the area controlled by each board. Within each Board's region sites were chosen to cover areas of low, medium and high susceptibility to acidification, based on solid geology.

### 2.2 Water Chemistry

Water samples were collected four times a year between March 1986 and December 1988 and analysed for the following determinands: pH, calcium, alkalinity, sulphate, chloride, conductivity and total filterable aluminium. Doughty (1989) gives details of the analytical methods used by the different purification boards for each determinand.

One of the aims of the water sampling was to characterise the mean conditions at a site. Determinations for each site were therefore screened to remove outliers. First, univariate outliers were removed by deleting samples in which any single determination was greater than three standard deviations from the mean for that site. Multivariate outliers were then removed by deleting samples whose score on either axis one or two of a principal components analysis of the log-transformed chemical data was more than three standard deviations from the mean score. Data screening removed about 6% of all determinations. For all subsequent analysis the chemical data were expressed as the arithmetic mean of undeleted samples.

### 2.3 Other Environmental Data

In addition to water chemistry various physical data were collected for each site. Catchment area, altitude, slope, and distance from source were determined from 1:50000 Ordnance Survey maps. Stream bed width was measured in the field for a typical reach at each site.

The percentage of catchment covered by mature conifer forest was estimated from maps, and converted to the following categories: 0 = 0%, 1 = <20%, 2 = 21-40%, 3 = 41-60%, 4 = 61-80%, and 5 = >81%.

Information on catchment solid geology was obtained from geological maps and classified according to the Geological Survey 'Ten Mile' map of Northern Britain. Doughty (1989) gives full details of the geology of each catchment and classifies each site according to the dominant rock type into one of the following: 1 = granites, 2 = Moine schists, 3 = Dalradian schists, slates etc., 4 = Ordovician & Silurian shales etc., 5 = basaltic and andesitic lavas, and 6 = limestones and base-rich sandstones.

### 2.4 Diatom analysis

Diatom samples were collected twice a year, in the spring (March/April) and late summer (August/September). At each site one to five cobble sized stones were selected from an area of 20 cm-30 cm water depth. Epilithic diatoms were removed by gently brushing with a toothbrush and transferred to a glass or plastic vial. Samples were preserved with formaldehyde or industrial methylated spirits within 8 hours of collection.

Samples were prepared using standard techniques Battarbee (1986). Approximately 250 valves were counted in each sample and diatom counts converted to percentages for all numerical analysis. Diatoms were identified using standard floras, especially those of Hustedt (1930-66), Germain (1981) and Kramer & Lange-Bertalot (19??), and unpublished taxonomic working paper produced by the Environmental Change Research Centre. Nomenclature follows Hartley (1986) and taxonomy follows that adopted by the Surface Water Acidification Programme (Stevenson et al., 1991).

### 2.5 Data analysis

Ordination

PCA

CCA

### Classification

The diatom samples were classified in two stages. First an initial classification was derived from a two-way indicator species analysis of the diatom percentage data using the program TWINSPAN (Hill 1979). Allocation of samples to groups was then refined to maximise within-group homogeneity while maintaining the hierarchical structure of the classification using the program FLEXCLUS (van Tongeren 1986). The program DISCRIM (ter Braak 1982) was then used to derive indicator species for the FLEXCLUS groups.

### 3 Site and catchment characteristics

### 3.1 Summary statistics

The location of the 149 sites is shown in Figure 1. Appendix 1 lists site codes, full names, grid references, together with site and catchment details. The majority of sites lie in areas classified as susceptible to acid deposition on the basis of solid geology (Kinniburgh and Edmunds, 1986), and most regions north of the Highland Boundary Fault and in the Southern Uplands are represented.



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Figure 2 shows frequency distributions of the main stream and catchment characteristics. The majority of sites (124) are situated in relatively small catchments of less than 100 km<sup>2</sup>, and lie within 30km of their source (mean distance to source = 8.7km). Many are low-lying, with only 26 sites situated above 300 m altitude. Stream width varies from 1 m to 50 m with a mean of 10.1 m.

Table 1 lists the number of sites on each geology type and Table 2 lists the number in each forestry class. The majority of sites (117) have less than 10% mature conifer forest in their catchment, and only 6 sites are more than 60% afforested.



# Figure 2 Frequency distributions of stream and catchment characteristics

Table 2Number of sites on each geology type

Geology type	Number of Sites
Granite	31
Moine schists	52
Dalradian schists, slates etc	26
Ordovician & Silurian shales etc	19
basaltic and andesitic lavas	6
limestones and	14

### 4 Water Chemistry

### 4.1 Summary statistics

Frequency distributions of chemical determinands is shown in Figure 3. Full mean chemistry for the three year sampling period is listed in Appendix 2.

Table 3 lists the numbers of sites in each of the three acidity categories identified by the UK Acid Waters Review Group (1986). Approximately  $1/4_{+}$  the sites (36) are classified as either permanently or occasionally acid.

Acidity Class	Number of Sites
Permanently Acid mean pH < 5.6, mean Alkalinity < 20 $\mu$ eq l <sup>-1</sup>	7
Occasionally Acid min pH < 5.6, mean Alkalinity < 20 $\mu$ eq l <sup>-1</sup>	29
Never Acid min pH > 5.6, mean Alkalinity < 100 $\mu$ eq l <sup>-1</sup>	113



Figure 3 Frequency distributions of chemical and physical determinands

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Table 3Number of sites in each-acidification class

Doughty (1989) discusses the relationships between pH and other chemical variables. These are presented here in Table 4 as correlation matrix, and in Figure 4 as a scatterplot matrix and summarised by a principal components analysis (PCA) of the log-transformed chemical data. Figure 5 presents the PCA ordination diagram, with the results plotted as a correlation biplot (ter Braak, 1987). In the ordination diagram sites are represented by points and variables by biplot arrows which point in the direction of maximum variance for that variable across the diagram. The angle between arrows reflects the correlation between variables, with small angles representing high positive correlation (Jongman et al, 1987). Axis one accounts for 61% of the total variance in the data and reflects the strong pH / alkalinity gradient, and the positive correlation between pH, alkalinity, calcium and conductivity. Low pH sites (e.g. 204, 612, 614) are identified on the left of the diagram. Axis two accounts for 17% of the variance and reflects essentially the total aluminium gradient, and the weak negative correlation between total aluminium and pH, with the high aluminium, low pH sites (e.g. 308, 204 and 304) plotted top left.



### Figure 4 Scatterplot matrix of physico-chemical variables

	pH	COND	CA	S04	ALK
PH	1.000				
COND	0.729	1.000			
CA	0.867	0.910	1.000		
S04	0.506	0.806	0.776	1.000	
ALK	0.950	0.783	0.919	0.592	1.000
AL	-0.514	-0.216	-0.305	-0.034	-0.409
CL	0.180	0.614	0.312	0.302	0.221
NM SO4	0.373	0.524	0.588	0.831	0.469
CATCH	-0.019	-0.033	-0.065	-0.236	-0.060
ALT	0.080	-0.116	0.058	0.057	0.147
SLOPE	0.016	-0.117	-0.028	0.035	0.032
WIDTH	-0.131	-0.151	-0.133	-0.222	-0.144
DIST SOU	-0.007	-0.058	-0.057	-0.233	-0.050
FORESTRY	-0.205	0.100	0.035	0.219	-0.075
C1	-0.379	-0.259	-0.339	-0.039	-0.403
C2	-0.195	-0.306	-0.351	-0.580	-0.235
C3	0.119	-0.060	0.124	0.154	0.136
C4	0.149	0.346	0.306	0.307	0.221
C5	0.236	0.154	0.186	0.133	0.185
C6	0.370	0.453	0.420	0.367	0.400
	AL	CL	NM_SO4	CATCH	ALT
AL	1.000				
CL	-0.006	1.000			
NM_SO4	0.032	0.012	1.000		
CATCH	0.020	0.196	-0.256	1.000	
ALT	0.192	-0.504	0.144	-0.330	1.000
SLOPE	0.002	-0.342	0.180	-0.642	0.357
WIDTH	0.087	0.021	-0.155	0.771	-0.215
DIST_SOU	-0.010	0.127	-0.211	0.947	-0.313
FORESTRY	0.327	0.172	0.184	-0.058	-0.077
	SLOPE	WIDTH D	IST_SOU F	ORESTRY	C1
SLOPE	1.000				
WIDTH	-0.536	1.000			
DIST_SOU	-0.598	0.739	1.000		
FORESTRY	-0.038	0.099	-0.061	1.000	

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Table 4

### Product-moment correlations between chemical and physical variables

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Figure 5 Principal components correlation biplot of log-transformed chemical data

### 4.2 The influence of site and catchment characteristics on water chemistry

The relationships between water chemistry, in particular pH, and geology type and percentage afforestation is discussed by Doughty (1989). The general pattern of these relationships is summarised here by a redundancy analysis (RDA) of the chemical, site and catchment variables. Figure 6 shows the RDA biplot of log transformed chemical and catchment variables (except pH-untransformed and altitude-square root transformed). Forestry was scored according to percent afforestation (see above), and geology type was entered as a series of dummy variables (ter Braak, 1987). The plot summarises the correlation structure between the chemical variables and the set of site physical and catchment variables. Sites are shown as points, chemical and quantitative catchment variables are shown as arrows. Dummy variables are plotted as points which are the centroids of their site scores (Jongman et al, 1987). Interpretation of the biplot arrows follows that for the PCA, with the angle between variables reflecting their correlation, and the length of an arrow reflecting the variance of that variable.



## Figure 6 Redundancy analysis biplot of the log-transformed chemical (dotted arrows) and catchment variables (solid arrows and triangles)

The catchment variables in the first two axes account for 39% of variance in the chemical data, and provide a convenient summary of catchment and site influence on the general chemical trends. As in the PCA the first axis reflects the pH / alkalinity gradient and the correlation between pH, calcium, alkalinty and conductivity, with high pH sites plotted on the right of the diagram, and low pH sites on the left. The influence of geology type is reflected by the position of the centroids for the six geology categories, with the 'sensitive' bedrock type of categories 1 and 2 (granites and Moine schists) plotted with the acid sites, and the 'less-sensitive' and 'non-sensitive' categories 4, 5 and 6 plotted with the high pH sites on the right of the diagram.

Axis two contrasts the higher altitude and steeper gradient sites with lower, afforested sites, and reflects the positive correlation between chloride and percentage afforestation. The tendency of steeper gradient sites to be located on geology types 3 and 5 is also shown. Biplot arrows also reflect the positive correlation between stream width, distance from source and catchement area and the negative correlation of the latter with altitude and slope. However these variables have relatively little variance and are not clearly related to stream chemistry.

To test the statistical significance of the effect of site and catchement variables on water chemistry the analysis was repeated using forward selection of explanatory variables. This is analogous to a stepwise multiple regression with explanatory variables entering the model only if they explain a significant amount of variance in the chemical data, as assessed using a Monte Carlo permutation test (ter Braak, 1991). Table 3 lists the results of the forward selection for variables explaining a significant amount of variance (p = 0.01). As expected the most important variable influencing overall stream chemistry is geology type. Figure 6 summarises the relationship between pH and rock type in a series of boxplots. Five of the seven permanently acid sites are situated on rock Type 1 (granite), while 58 of the 71 occasionally acid sites are located on Type 1 or Type 2 (Moine schists). Sites located on the Ordovician and Silurian shales of Type 4 are clearly the most variable, including some of the most acid and most alkaline sites. Sites situated on Type 5 (basaltic and andesitic lavas) or Type 6 (limestones and sandstones) bedrock have a similar mean pH range but are clearly separated on the RDA biplot, with Type 6 sites generally having higher alkalinities and conductivities.

### 5 Epilithic Diatoms

### 5.1 Species occurrence and abundance

Seven hundred and ten diatom samples were collected from the 149 sites, containing a total of 215 taxa. Many of these taxa were rare, occurring in only one or two samples, and provide little ecological information. Infrequent taxa were therefore removed and the dataset reduced to 134 taxa by excluding those that occurred in less than 5 samples, and achieved a maximum relative abundance of at least 1.0 percent in any single sample. Appendix 3 gives a full list of these taxa and authorities, together with maximum abundance and number of occurrences.

The relationship between abundance and occurrence is shown in Figure 7. One taxa, *Achnanthes minutissima* is found at most sites and dominates the assemblage at many. Other widespread and abundant taxa are plotted on the top right of Figure 7. Taxa which are common but less widespread are plotted top centre, and include *Hannaea arcus* and *Eunotia exigua*. The majority of taxa may be classed as local and rare (bottom left of Figure 7) but there are a number of locally abundant forms (plotted top left), including *Peronia fibula* and *Achnanthes saxonica*.

The number and distribution of taxa within each sample may be summarised using a measure of sample diversity. An intuitive measure is simply the number of taxa in a sample, but this



### Figure 7 Relationship between number of occurrences and maximum abundance

may be dependent on sample size - as more individuals are counted, more taxa are found. A convienient diversity index which is less sensitive to sample size is Hill's N<sub>2</sub> (= reciprocal of Simpson's index), which gives a measure of the effective number of taxa in the assemblage (Hill, 1973). Figure 8 summarises the distribution of sample diversities. The majority of samples have an effective number of taxa of less than 10, and 65% have less that four. Twenty-two percent of the samples many may be considered species poor with  $N_2$  less than two. As expected, the epilithon samples described here have a lower diversity that lake sediment diatom assemblages from similar water chemistry ( $N_2$  typically 5 - 20), with the latter composed of diatoms from a variety However they are similar to of habitats. epilithic diatom assemblages from other fresh waters (Juggins, 1989; Stevenson et al. 1991).



Figure 8 Frequency distribution of  $N_2$ sample diversity



Figure 9 Relationship between  $N_2$  sample diversity and pH

Biological diversity is often used as an indicator of water quality (see discussion in van Dam, 19??). However in this dataset there is no significant relationship between diversity (Hill's  $N_2$ ) and pH (Figure 9), or other chemical or catchment variables.

Figures 7 and 8 indicate that many samples are dominated by a small number of very abundant taxa which occur across a wide range of environmental conditions. However, as Figure 9 shows, the majority of taxa have a more restricted distribution across samples, and hence water quality types.

### 3.2 Sample classification

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The TWINSPAN/FLEXCLUS sample classification and main indicator species derived from DISCRIM are shown in Figure 10. Figure 11 summarises the classification, showing the mean abundance of common species in each sample group. Eight major sample groups were identified at three levels of division and 18 subgroups identified at 5 levels.

The first dichotomy separates sample groups 1-5 from groups 6-8, with *Tabellaria flocculosa* and *Brachysira vitrea* as negative indicators (ie. differential species for groups 1-5), and *Gomphoneis olivaceoides*, *Fragilaria vaucheriae*, *Cymbella ventricosa* and *Hannea arcus* as positive indicators.





### Figure 10 TWINSPAN/FLEXCLUS sample classification, showing indicator taxa for each dichotomy

negative indicators (resulting in groups 1 and 2), and Achnanthes minutissima and Synedra miniscula as positive indicators (resulting in groups 3-6). Division 3 resulted in the separation of group 6 samples, dominated by Achnanthes minutissima from group 7 and 8 samples, characterised by Cymbella ventricosa, Cymbella minuta, Cocconeis placentula var. euglypta



and Gomphoneis olivaceoides.

Division 4 included Eunotia exigua and Eunotia incisa as negative indicators and separated the Eunotia exigua dominanted samples of group 1 from the Tabellaria flocculosa dominated samples of group 2. Division 5 separated the Achnanthes saxonica and Gomphonema parvulum dominated assemblages of groups 3 and 4 from those of group 5, characterised by Brachysira vitrea, Achnanthes minutissima, and Tabellaria flocculosa. Division 6 split the Achnanthes minutissima dominated assemblages of group 6 into a group characterised by virtually monospecific assemblages of Achnanthes minutissima (6A), and a higher diversity group characterised by the occurrence of Fragilaria vaucheriae and Synedra miniscula (6B). Division 7 had Hannae arcus, Gomphoneis olivaceoides and Fragilaria vaucheria as negative indicators (resulting in group 7) and Cocconeis placentula var. euglypta, Navicula avenacea, Navicula gregaria and Nitzschia palea as positive indicators (resulting in group 8).

Division 8, at level 4, divided the *Tabellaria flocculosa* dominated samples of group 2 into a low diversity group (2B), and a higher diversity group (2A) characterised by the the occurrence of *Eunotia exigua*, *Eunotia incisa*, *Peronia fibula* and *Frustulia rhomboides* var. *saxonica*. Division 9 separated the *Achnanthes saxonica* dominated assemblages of group 3 from those of group 4, characterised by *Gomphonema parvulum*. These two groups were further subdivided at divisions 13 and 14, with group 3 samples subdivided into those exclusively dominated by *Achnanthes saxonica* (bgroup 3A) and those also containing significant numbers of *Achnanthes detha* and *Cymbella lunata* (group 3B), and group 4 samples subdivided into those characterised by *Pinnularia subcapitata* var. *hilseana* and *Pinnularia irrorata* (group 4A) and those characterised by *Meridion circulare* (group 4B).

The Achnanthes minutissima/Tabellaria flocculosa/Brachysira vitrea assemblages in group 5 were further subdivided into three subgroups at levels 4 and 5 (divisions 10 and 15 respectively), with group 5A characterised by Eunotia exigua, Eunotia pectinalis fo. impressa, group 5B by Synedra acus and Cymbella microcephela, and group 5C by Navicula [sp1 cf. tantula], Achnanthes detha and Achnanthes biasolettiana.

Groups 7 and 8 could each be similarly subdivided into three subgroups at levels 4 and 5. Division 11 separated group 7A, with *Hannea arcus* and *Diatoma hyemale* var. *mesodon* as negative and *Cymbella* species as positive indicators. Division 16 separated samples of group 7B, characterised by *Fragilaria vaucheriae* and *Cymbella minuta*, from those of group 7C, characterised by *Gopmphonema clevei* and *Gomphoneis olivaceoides*. Group 8 were subdivided at divisions 12 and 17, to form a *Cocconeis placentula* var. *euglypta* dominated group (group 8A), a *Navicula avenacea* and *Navicula gregaria* dominated group (group 8B), and a *Nitzchia palea* and *Nitzschia palea* var. *tenuirostris* dominated group (group 8C).

GRO	N	Season	pH		Conductiv	ity	Calcium		Sulphate		Alkalinity		Total Alum	inium	Chloride	
			mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
1	20	0.45	5.20	0.49	41.7	4.9	59.3	19.2	110.0	19.5	8.2	13.8	157.0	56.4	173.0	47.4
2A	53	0.55	5.99	0.44	36.1	12.1	54.8	23.6	68.6	29.6	28.4	31.1	64.2	28.2	175.8	72.7
2B	43	0.42	6.01	0.38	34.1	9.6	54.4	14.1	65.0	21.1	26.9	21.2	64.4	29.7	159.4	80.9
ЗA	15	0.53	6.19	0.52	53.8	9.7	108.2	46.3	87.4	13.1	60.2	55.0	93.9	52.4	276.1	48.0
3B	7	0.29	6.40	0.26	68.3	14.9	172.4	74.9	115.9	. 29.1	85.5	36.9	107.2	51.7	341.7	83.5
4A	23	J.65	6.28	0.31	56.0	18.0	124.5	62.9	90.1	27.5	76.6	28.4	119.4	58.2	285.3	113.8
4B	13	1.00	6.45	0.30	53.2	16.7	99.3	34.7	66.9	18.0	105.4	59.2	71.5	37.8	272.2	119.3
5A	124	0.39	6.49	0.22	40.1	12.4	92.5	50.5	60.3	31.2	77.1	55.2	54.5	25.9	181.0	76.5
5B	81	0.32	6.59	0.31	44.2	10.6	113.8	67.6	57.0	26.2	93.8	81.5	49.5	18.3	205.6	102.7
5C	10	0.30	6.60	0.20	70.2	10.7	127.1	13.8	70.1	20.9	142.6	57.3	63.9	23.1	409.1	109.2
6A	37	0.49	7.01	0.35	54.8	18.3	221.3	110.2	91.7	28.6	247.0	185.3	43.1	22.2	155.9	69.4
6B	116	0.41	6.91	0.36	56.9	27.5	207.2	161.5	83.3	42.3	232.7	228.9	52.7	25.7	176.3	113.5
7A	55	0.86	7.18	0.37	77.6	37.8	343.7	225.6	126.3	67.6	416.5	325.1	46.0	23.1	166.6	104.2
7B	25	0.24	7.50	0.32	113.8	46.4	571.0	280.1	159.2	75.3	751.1	435.0	38.8	17.6	185.1	72.9
7C	27	0.89	7.60	0.37	136.6	65.1	712.0	500.2	187.8	113.0	906.4	576.5	51.4	26.2	250.4	132.7
8A	38	0.18	7.47	0.32	123.5	64.4	594.3	429.2	171.0	109.2	747.0	497.2	43.4	18.9	260.1	147.5
8B	20	0.75	7.73	0.29	257.6	170.9	1309.8	1007.8	437.7	372.3	1524.0	1089.5	48.2	13.3	446.4	310.8
8C	7	0.00	7.76	0.30	304.6	191.5	1566.1	1156.7	472.4	457.5	1887.6	1229.8	36.9	22.6	526.0	344.9

5 Mean chemistry for each diatom group

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Table 5

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the ph / water hardness gradient, and a strongly arched second axis, even with detrending (Jongman *et al.* 1987), making the ordination difficult to interpret. The dataset was therefore divided into two and sample groups 1-5 and 6-8 analysed separately.

### Groups 1-5

With season as a covariable and using forward selection of the remaining environmental variables the following were significant in the 1-5 group dataset (p = 0.01, Monte Carlo permutation test, 99 permutations): pH, alkalinity, conductivity, calcium, sulphate, alkalinity, total aluminium, chloride, catchment area, catchment afforestation, distance from source, and geology types 1 and 2. Monte Carlo permutation tests (99 permutations) of axis 1 and of axis 2 with axis 1 as an additional covariable show that both axes were significant (p = 0.01). Figure ?? shows the position of the most common taxa and sample group centroids on axes 1 and 2, ( $\lambda_1 = 0.33$ , 4.5 standard deviation units,  $\lambda_2 = .32$ , 5.0 standard deviation units). Non-significant environmental variables were added to the ordination as passive variables (ter Braak, 1988) and are enclosed in brackets in Figure ??.

Inter-set correlations between the environmental variables and ordination axes show that axis 1 is a gradient of pH (r=0.74), alkalinity (0.75), and calcium (0.56). The ordering of sample groups along axis one essentially follows that of the classification, with group 1 samples from the most acid sites on the left, groups 2A, 3B and 3A in the centre and groups 3B-5C, mainly from sites with a pH greater than 6.5, on the left of the diagram. The position of taxa along axis 1 approximates their pH optima (Jongman *et al.* 1987), with acidobiontic taxa (eg. *Tabellaria quadriceptata* - TA004A and *Eunotia incisa* - EU047A) plotted on the right of the diagram, acidophilous forms (eg. *Tabellaria flocculosa* - TA001A and *Frustulia rhomboides* var. *saxonica* - FU002B) plotted centre, and circumneutral taxa (eg. *Achnanthes minutissima* - AC013A and *Cymbella minuta*) plotted right. The centre of the diagram corresponds to a pH of ca. 6.3, the mean of the dataset.

Axis 2 reflects catchment afforestation (inter-set correlation=0.58), with groups 3 and 4, containing samples derived from circumneutral, mainly afforested sites, plotted at the top of the diagram and samples from unafforested sites, classified into groups 2 and 5, plotted bottom. Taxa with a pH optimum of less than ca. 6.3, the mean of the dataset, are plotted to the right of centre and axis 2 contrasts those common at afforested sites, (eg. Achnanthes saxonica - AC028A, Pinnularia subcapitata var. hilseana and Achnanthes austriaca var. helvetica - AC014C), with those dominant at unafforested sites (eg. Tabellaria flocculosa - TA001A, Peronia fibula - PE002A, and Brachysira brebessonii - BR006A). To the left of the diagram, taxa with a pH optima greater than 6.3 and common at afforested sites, (eg. Achnanthes detha - AC042A, Navicula [sp1. cf. minima] - NA086A, and Gomphonema parvulum - GO013A) are contrasted with those common in samples from unafforested sites (eg. Brachysira vitrea - BR001A, Hannea arcus - HN001A, and Cymbella microcephela - CM004A).

An third major gradient, that of total aluminium, is also identified in figure ??. This runs at an angle of ca. 45° to axis 1, with values increasing towards the top right. Total aluminium is inter-correlated with pH and afforestation, and samples from the low pH sites (group 1) and those from afforested sites (groups 3 and 4) have high total aluminium concentrations (Figure ??). These groups are positioned at the high end of the gradient and contrasted with samples from the unafforested, higher ph sites, with low total aluminium (groups 2 and 5), at the lower end. Taxa plotted top right (eg. Achnanthes austrica var. minor -AC014B, Achnanthes

## 3.3 Relationship between sample groups and stream water chemistry / catchment characteristics

Table 5 lists mean chemical and catchment characteristics and Table ?? lists afforestation status for each sample group. Figure 12 presents box-plots summarising the distribution of pH across the groups. Table ?? and Figure 12 clearly show that the sample classification is strongly related to the water hardness-pH gradient, with the major divisions separating samples containing either (i) acidiobiontic and acidiphilous species, indicative of acid waters (group 1), (ii) acidiophilous and circumneutral species, indicative of circumneutral waters, (groups 2-5), or (iii) circumneutral and alkaliphilous taxa, indicative of waters with pH greater than 7 (groups 6-8).

In addition to the chemical gradient there is significant variation in the diatom assemblages due to the season of sampling and while many of the assemblages were recorded in both spring / early summer and late summer / autumn some were restricted to a single season. For example, the *Gomphonema parvulum* dominated assemblages of group 4B were only found in the late summer / autumn while the *Nitzchia palea* assemblages of group 8C were confined to the spring / early summer. Other groups were not so restricted to a single season but did show a preference, with groups 7A, 7C and 8B predominating in the late summer / autumn and groups 7B and 8A predominating in the spring / early summer.

To remove the effect of seasonality and focus on the relationships between the diatom assemblages and chemical and catchment characteristics a partial canonical correspondence analysis of the joint diatom and environmental data was performed, with season as a covariable. Initial analysis of all samples produced an ordination with a first axis reflecting



Figure 12 Boxplot showing distribution of pH across diatom groups



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austriaca var. helvetica, Eunotia exigua and Pinnularia subcapitata var. hilseana) are identified as aluminium tolerant.

### Groups 6-8

In a similar analysis of sample groups 6-8 the following environmental variables were significant (p = 0.01): pH, alkalinity, conductivity, calcium, sulphate, total aluminium, chloride, stream width, catchment afforestation, altitude, and geology types 1 to 4. Both axes were significant (Monte Carlo permutation tests, 99 permutations). Figure ?? shows the position of the most common taxa and sample group centroids on axes 1 and 2, (la1 = 0.33, 4.0 standard deviation units, la2 = 0.12, 4.4 standard deviation units).

Again the main direction of variation in the data is along the pH / hardness gradient, lying at an angle of ca. -20° to axis 1, with pH increasing from left to right. The ordering of sample groups along this gradient approximately follows the TWINSPAN classification from groups 6A and 6B plotted top left to group 8C plotted bottom right. Similarly acidophilous taxa (eg. *Tabellaria flocculosa* - TA001A and *Eunotia pectinalis* var. *impressa*) are plotted top left, circumneutral species (eg. *Achnanthes minutissima* and *Cymbella minuta*) plotted centre, and alkaliphilous taxa (eg. *Navicula avenacea* and *Nitzschia palea*) are plotted bottom right. A number of taxa brackish-water tolerant taxa common in group 8 samples from high conductivity sites (eg. *Rhoicosphenia curvata*, *Navicula gragaria* and *Navicula gracilis*) are also plotted bottom right.

A second gradient relating to altitude and stream width / catchment area runs at about -135° to axis 1, from higher altitute, smaller catchment and stream width sites in the bottom left to samples from lower altitute, wider streams, with larger catchments in the top right.

### 5.4 Site classification

If the composition of the diatom assemblages at a single site remained unchanged throughout the three year sampling period then the sample classification derived in above could equally well be used to classify sites. However, as Appendix ?? shows, the constancy of diatom assemblages varies considerably at a single site, from sites where the same asemblage type is found at each sampling time, to sites where a different assemblage was recorded from each sample. A classification of sites, on the basis of the assemblages types defined above, was performed by a ?????? clustering of the Euclidean distance matrix derived from the site by sample group table.

Twelve site groups were delimited in the site classification, each characterised by one or more assemblage types. The allocation of sites to sites groups is listed in Appendix ?? and Table ?? lists the numbers of samples of each assemblage type occurring in each site group. Figure ?? shows a map of sites, with each site indicated by its group number.

The relationship between site groups, and environmental variables is summarised by axes 1 and 2 of a canonical variates analysis shown in Figure ??. In the diagram sites are shown as group centroids, and environmental variables as arrows, with assemblage types added as the centroids of the sample in which they occur. Interpretation is similar to that for the CCA diagrams, with assemblages occurring frequently at sites in a particular site group plotted close to that group.



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Figure 14 CCA ordination diagram of diatom groups 6-8

Table ?? and Figure ?? show that the site classification generally follows the sample classification, and that the site groups are strongly related to the environmental gradients revealed by the CCAs, namely ph / hardness, conductivity, aluminium and forestry.

- Site group 1 (n=5): Afforested, poorly buffered, low pH sites with high total aluminium. Characterised almost exclusively by the *Eunotia exigua* dominated assemblage of sample group 1.
- Site group 2 (n=16): Non- and lightly afforested, poorly buffered, circumneutral sites, characterised almost exclusively by the *Tabellaria flocculosa* dominated assemblages of sample groups 2A and 2B.
- Site group 3 (n=3): Afforested, moderately well buffered circumneutral sites, characterised almost exclusively by the *Achnanthes saxonica* dominated assemblage of sample group 3A.
- Site group 4 (n=12): Lightly to heavily afforested, moderately well buffered, circumneutral sites with high total aluminium. Characterised by assemblage types 4A (both seasons), 4B (late summer / autumn), and 5C (spring / early summer), together with occasional samples of assemblage types 2, 3 or 5A.
- Site group 5 (n=18): Non- or lightly afforested, moderately to well buffered, circumneutral sites with low total aluminium. Characterised almost exclusively by assemblage type 5A.
- Site group 6 (n=22): Non- or lightly afforested, moderately to well buffered, circumneutral sites with low total aluminium. Characterised by assemblage types 5A and 5B, with occasional samples of types 2A, 6A or 6B.
- Site group 7 (n=8): Non- or lightly afforested, moderately to well buffered, circumneutral sites with low total aluminium. Characterised almost exclusively by assemblage type 5B, with occasional samples from type 5A.
- Site group 8 (n=27): Well buffered, ca. pH 7.0 sites with relatively large catchments (mean 58 ha) and little or no catchment afforestation. Characterised predominantly by type 6B assemblages, together with types 5A and 5B (both seasons), and 7A (late summer / autumn).
- Site group 9 (n=9): Well buffered, ca. Ph 7.0 sites with small catchments (mean 15.4 ha) and little or no catchment afforestation. Characterised by a mixture of assemblage types 6A and 6B, with occasional samples of type 7A (late summer / autumn).
- Site group 10 (n=12): Sites with slightly alkaline waters situated in small, higher altitude catchments (mean area 7.3 ha, mean altitude 212 m), with little or no afforestation. Characterised by assemblage types 7A and 7B, with occasional occurrence of types 6B (spring / early summer) and 7C (late summer / autumn).
- Site group 11 (n=14): Sites with alkaline waters and moderately high conductivities (mean  $135 \,\mu\text{S cm}^{-1}$ ) situated in large catchments (mean 144 ha) with little or no afforestation.

Characterised by assemblage types 8A (spring / early summer) and 7C (late summer / autumn), with occasional occurrence in the late summer / spring of types 7A, 8A and 8B.

Site group 12 (n=3): Sites with very high conductivity, alkaline waters (mean Ph 7.9, mean conductivity 409  $\mu$ S cm<sup>-1</sup>), situated large catchments (mean 101 ha) with little or no afforestation. Characterised by assemblages 7C, 8B and 8C (spring / early summer) and 8B (late summer / autumn).

### Appendix 1 Site codes, locations and catchment characteristics

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Site Code	Site Name	Location	Gric	Ref		Catchment Area (km)	Altitude (m)	Slope	Width	Distance from source	Afforestation
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101	Allt Eigheach	B846 Rd Bridge	NN	442	577	27.4	260	25	9	12.0	0
102	Allt Lairig nan Lunn	Upstream of dam	NN	451	412	6.2	370	29	4	6.2	0
103	River Cononish	Upstream of confluence	NN	340	289	35.5	170	10	18	9.5	1
104	Water of Mark	Rd Bridge at Invermark	NO	444	804	48.6	250	13	12	18.3	. 0
105	West Water	Rd Bridge at Waterhead	NO	465	716	40.5	290	20	13	11.7	0
106	White Water	Forestry Comm Rd Br	NO	275	762	25.9	270	33	7	8.4	1
107	Lochbroom Burn	Br at Milton of Dalcapon	NN	979	550	9.7	190	50	3	7.7	1
108	Keltney Burn	Rd Br at Keltneyburn	NN	774	491	45.6	100	31	9	14.0	1
109	Urlar Burn	Car Par at Aberfeldy	NN	855	486	29.1	130	67	6	9.5	1
110	Ballinloan Bum	Wade Bridge at Ballinloan	NN	974	405	36.0	150	31	6	11.1	2
111	White Burn	Rd Br Newmill of Inshewan	NO	423	609	8.5	180	20	3	5.4	1
112	Shaggie Burn	Ft Br u/s Rd Br at Monzie	NN	878	251	11.5	110	29	4	7.3	1
113	Edendon Water	Upstream Edendon Br over A9	NN	716	706	140.0	150	6	20	32.0	0
114	Drumturn Burn	Rd Br near Blackall Farm	NO	142	566	10.9	230	95	3	6.9	1
115	Pow Bum	Rd Br at Powmouth	NO	651	577	60.0	5	2	5	12.1	2
116	Slack Burn	Clattering Bridge	NO	665	783	7.1	130	20	2	4.8	1
117	Glassart Bum	Rd Bridge	NO	229	135	6.7	90	13	2	3.3	2
118	Water of May	Rd Br at Pathstruie	NO	76	119	15.7	130	15	4	9.7	2
201	Brioch Burn	u/s Upper Glendevon Reservior	NN	914	36	6.3	335	41.6	З	3.7	0
202	Ardoch Burn	At the Bows	NN	741	63	20.4	168	20.8	5	8.6	1
203	Keltie Water	At Arivurichardich	NN	640	138	9.8	290	18.5	5.5	5.6	0
204	Kelty Water	At Clashmore	NS	467	968	2.8	168	20.8	З	2.7	3
205	Tighanes Burn	u/s from Strathyre	NN	563	168	3.4	152	167.5	3.5	2.4	4
206	Casaig Burn	u/s Glen Finglas Reservoir	NN	532	93	8.2	183	167.5	6	4.3	0
207	Gleann Riabhach Burn	u/s Keepers Cottage	NN	502	62	7.6	91	33.4	5	5.0	3
208	Unnamed Burn	Ft Br u/s from Loch Chon	NN	410	67	3.4	107	83.3	5	2.4	3
209	Boquhan Burn	u/s from Pow Burn	NS	653	922	5.0	122	27.8	3	4.4	0
301	Main Water of Luce	Above Penwhim Burn	NX	137	697	32.0	110	10	10	11.0	1
302	Cross Water of Luce	Near Barnshangan	NX	193	651	61.0	70	10	15	19.0	1
303	Tarf Water	Tarf Bridge	NX	255	648	38.0	100	10	15	13.0	3
304	River Bladnoch	Glassoch	NX	333	696	54.0	60	2.5	15	14.0	4

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305	Water of Minnoch	Above Water of Trool	NX	371	785	81.0	70	30	20	17.0	3
306	Water of Trool	Above Water of Minnoch	NX	378	782	47.0	60	5	12	10.0	3
307	Cooran Lane	Above Brishie Burn	NX	478	833	16.0	250	10	7	5.0	3
308	Black Water of Dee	Below Struan Loch	NX	654	692	74.0	70	10	20	30.0	4
309	Crae Lane	Above Black Water of Dee	NX	664	688	26.0	60	2	12	8.0	3
310	Scar Water	Below Chanlock Burn	NX	792	999	49.0	150	10	20	13.0	1
311	Glensome Burn	Above Solway Fishery	NX	938	663	18.0	50	10	8	6.0	3
312	Urr Water	At A712 Rd Br	NX	766	758	79.0	100	5	15	12.0	2
313	Water of Milk	Nr B7068 Rd Br	NY	163	814	101.0	80	10	20	16.0	1
314	Liddel Water	Above Hermitage Water	NY	502	896	85.0	110	3.3	20	20.0	2
401	Tweed	Tweedshaws	NT	52	150	1.6	400	20	10	1.2	1
402	Biggar	Whiterig	NT	128	348	129.0	200	10	8	18.5	1
403 '	Tweed	Above Gala	NT	509	347	1510.0	100	З	50	115.5	1
404	Southey Burn	Foot	NT	190	301	1.0	355	150	2	1.0	1
405	Lewinshope Burn	Old Tinnis	NT	389	295	8.3	235	22	4	5.4	1
406	Flosh Burn	Foot	NT	190	301	3.1	290	90	2	2.0	1
407	Whiteadder	u/s Reservoir	NT	642	650	11.9	265	8	5	4.9	0
408	Cockburnlaw Burn	Cockburn Law	NT	769	590	0.5	160	150	1	1.0	0
409	Leet Water	Coldstream Gauge	NT	839	396	113.0	8	1	7	21.1	0
501	North Sannox Burn	A841 Bridge	NR	994	468	11.7	50	39.5	6	4.4	0
502	Easan Biorach	A841 Bridge	NR	943	498	8.6	10	45.5	6	4.4	0
503	Catacol Burn	A841 Bridge	NR	911	489	12.3	5	19.5	7	5.3	0
504	lorsa Water	Dougarie Lodge	NR	884	370	50.5	5	20.3	19.5	13.5	0
505	Machrie Water	Monyquil	NR	937	349	21.4	60	8.8	12	5.4	0
506	Black Water	Pien	NR	919	304	10.4	50	44.1	12	6.2	3
507	Cloy Burn	u/s of Kilmichael	NS	1	348	7.7	30	18.5	6	4.0	2
508	Carrick Lane	u/s of Loch Doon	NX	476	941	51.1	220	12.1	14	10.7	2
509	Gala Lane	u/s of Loch Doon	NX	482	926	22.0	220	15.6	10	8.4	3
510	Garpel Burn	u/s of Loch Doon	NX	481	985	13.3	220	22.1	6.5	5.4	1
511	Water of Girvan	u/s of Loch Doon	NX	406	954	4.4	340	16.7	3.5	3.2	1
512	River Stinchar	High Bridge	NX	396	956	4.4	351	55.6	4	3.2	1
513	Palmullan Burn	Linfern	NS	382	16	13.2	137	25.4	8.5	7.3	1
514	Allt A'Bhiorain	Gualachulain	NN	113	455	7.8	5	83.3	7	4.7	3
515	Allt Nan Gaoirean	Glen Etive	NN	141	475	7.7	30	50	6.5	5.4	3
516	Allt Charnan	Inverchaman	NN	144	484	7.7	46	32.6	5	5.1	1
517	Allt Dubh	u/s Inverawe	NN	25	317	0.8	46	30	1.5	1.7	5
518	Linne Nam Beathach	Victoria Bridge	NN	271	422	50.1	168	9.8	26.5	12.5	1

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519	River Etive	Kingshouse Hotel	NN	260	547	18.1	240	12.8	15	8.2	0	
520	Allt Fhaolain	Inbhirfhaolain	NN	158	509	5.8	85	8	4	3.6	1	
521	Allt Tolaghan	A8005 Bridge	NN	271	413	11.6	168	7.4	8.5	6.9	0	
522	Water of Tulla	A82 Bridge	NN	314	444	48.2	168	6.8	21.5	16.1	- 1	
523	River Lochy	Glenlochy	NN	254	296	20.5	199	9.3	9	7.5	2	1
524	Allt Cruiniche	A85 Bridge	NN	37	294	1.9	46	150	4	2.7	0	ł.
525	River Lonan	d/s of Clachadow	NM	937	280	12.7	61	12.1	6	7.0	1	
526	Teatle Water	A819 Bridge	NN	124	251	16.6	46	17	8.5	9.1	1	
528	Dearg Abhainn	u/s Barcaldine House	NM	965	411	8.9	30	29.4	7	5.2	2	
529	River Nant	Forest Trail	NN	19	273	32.3	61	75	8	9.5	- 1	
602	Allt Darrarie	Spittal of Glenmuick	NO	309	852	13.0	400	100	5.5	6.0	0	
603	Feith Talagain	u/s Garvabeg	NN	525	959	18.2	320	80	11.1	6.5	0	
604	Allt Na Fearna	u/s Bhran Cottage	NN	754	911	9.3	360	33	4	6.0	0	
605	Callater Burn	Auchallater	NO	155	883	34.0	360	29	8	12.0	0	
606	Water of Feugh	Ballochan	NO	525	904	21.0	230	17	7	5.0	0	
607	Baddoch Burn	d/s Baddoch	NO	134	833	23.0	410	18	8	8.0	0	
608	Quoich Water	Quoich Cottage	NO	118	912	60.0	330	28	22	15.0	1	
609	Allt Na Baranachd	u/s Dunachton	NH	822	51	11.0	310	100	4.9	5.0	1	
610	River Muick	Loch Muick Outlet	NO	300	842	38.0	390	3	11	11.0	0	
611	Slugain Burn	Balnagower	NO	159	932	15.0	330	40	4	5.5	1	
612	Allt Dubh Loch	Dubh Loch Outlet	NO	265	821	13.0	410	40	6	6,5	0	
613	Allt Coire Chaoil	u/s Carnachuin	NN	847	934	6.7	350	66	2.7	3.5	0	
614	Glas Allt	Glas Alt Shiel	NO	267	825	5.0	400	100	3.5	5.0	1	
615	River Lui	u/s Dee confl.	NO	69	898	61.0	350	25	22	17.0	1	
616	Tullich Burn	Milton of Tullich	NO	387	975	16.0	200	44	4.5	7.5	0	
617	Allt Mor	Pitmain Lodge	NH	749	29	13.6	330	50	4.6	6.0	0	
618	Allt Fheamagan	Achleum	NN	851	971	7.7	320	36	З	4.0	1	
619	Cowlatt Bum		NJ	173	448	30.0	320	25	9	10.0	4	I
620	Allt A'Mharcaidh	u/s Lagganlia	NH	879	50	10.1	280	50	4.4	5.5	1	
621	Allt Ant-Slugain Dhuibh	Dalnahaitnach	NH	855	201	5.9	310	33	2.3	4.0	0	
622	Allt Ruadh	u/s Balachroick	NH	859	10	10.7	330	100	3.8	5.0	1	
623	Torwinny		NJ	133	485	50.0	300	20	12	12.0	5	
624	Wells of Lecht Burn	Picnic Site	NJ	235	154	5.1	450	33	2.3	2.5	0	
625	Allt Arder		NJ	173	417	48.0	350	20	22	10.0	0	
701	River Strathy	Strathy	NC	835	649	112.0	1	10	10	25.0	2	
702	River Strathy	u/s Nature Reserve	NC	803	523	27.0	140	5	3	8.0	1	
703	Achriesgill Burn	u/s B801	NC	257	541	26.0	10	40	3	3.0	0	

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705	Strath Burn	Strath	ND	255	521	65.0	35	8	4	20.0	0
706 '	River Laxford	d/s L More	NC	295	400	45.0	40	10	8	11.0	0
707	River Halladale	Forsinain	NC	904	485	85.0	70	10	20	15.0	1
708	Sleach Water	u/s Loch More	ND	54	461	67.0	120	10	З	15.0	0
709	River Mudale	u/s Altnaharra	NC	568	359	113.0	75	3.3	15	15.0	1
710	River Naver	d/s L. Naver	NC	676	387	366.0	65	5	30	30.0	1
711	Allt Nam Albannach	A838 Rd Br	NC	385	331	20.0	120	20	4	12.0	0
712	River Fiag	A838 Rd Br	NC	467	205	66.0	100	66.7	5	18.0	1
713	Traligill Burn	Inchnadamph	NC	250	218	20.0	70	28.6	6	6.0	0
714	River Helmsdale	Kildonan Lodge	NC	901	233	409.0	70	10	25	33.0	1
715	River Oykel	Suspension Bridge	NC	352	28	131.0	55	5.7	30	22.0	1
716	River Cassley	Glencassley Castle	NC	438	78	154.0	55	4	20	25.0	1
717	River Carron	Croik	NH	461	912	52.0	100	10	6	25.0	0
718	River Gruinard	u/s A832	NG	962	912	153.0	20	6.7	15	30.0	0
719	River Vaich	u/s A832	NH	373	709	15.0	210	8	10	16.0	0
720	River Blackwater	Inchlumpie	NH	589	749	171.0	160	13.3	6	12.0	2
721	Abhainn Bruachaig	Incheril	NH	38	624	94.0	30	5	8	16.0	0
722	River Glass	Eileannach Lodge	NH	545	689	72.0	210	8	7	20.0	1
723	River Meig	Bridgend	NH	321	550	150.0	100	8	9	30.0	0
724	River Orrin	Fairburn	NH	454	524	129.0	120	20	8	31.0	1
725	River Carron	New Kelso	NG	940	425	138.0	2	4	10	25.0	1
726	River Farrar	u/s Struy Bridge	NH	401	403	164.0	50	4.4	15	40.0	1
727	River Elchaig	Killilin	NG	942	299	105.0	1	2.5	11	17.0	0
728	River Naim	Balnafoich	NH	686	353	110.0	180	2.9	20	20.0	1
729	River Sligachan	d/s Motel	NG	487	300	35.0	1	25	6	10.0	0
730	River Findhorn	Tomatin Old Bridge	NH	804	278	270.0	300	4	30	38.0	1
731	River Shiel	Battle of Gleashiel	NG	995	134	22.0	60	6.7	3	9.0	0
732	Aldernaig Burn	A87 Bridge	NH	298	11	18.0	50	66.7	1	8.0	0
733	Allt A'Choire Beithe	u/s L Quoich	NG	985	36	6.0	210	5	2	2.0	0
734	River Oich	u/s A82 Bridge	NH	337	36	430.0	40	4	45	50.0	1
735	River Pean-Dessary	Strathan	NM	979	912	58.0	40	6.7	10	- 10.0	1
736	River Arkaig	d/s L Arkaig	NN	173	885	244.0	50	13.3	35	29.0	0
737	Allt Lon A'Mhuidhe	u/s L Eilt	NM	845	815	4.0	40	40	2	2.0	0
738	River Ailort	d/s L Eilt	NM	775	830	33.0	30	8	8	10.0	0
739	River Moidart	Brunnery	NM	730	719	33.0	10	6.7	10	10.0	0
740	River Callop	d/s A830 Bridge	NM	925	793	19.0	10	5	10	6.0	2

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741	River Finnan	u/s A830 Bridge	NM 907 808	26.0	10	13.3	8	9.0	0
742	River Shiel	Shielfoot	NM 665 704	247.0	2	2	35	40.0	1
743	River Nevis	Polldubh	NN 144 686	50.0	40	5	30	15.0	1
744	River Blackwater	u/s R Aline Confl	NM 702 506	31.0	20	66.7	6	14.0	0
745	River Aline	d/s L Arienas	NM 697 495	102.0	10	5	12	16.0	1
746	River Scaddle	Aryhoulin	NN 13 689	46.0	10	6.7	10	18.0	0
747	River Leven	u/s Brit Alcan	NN 195 618	182.0	10	40	25	30.0	0

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