## ECRC Research Report # 150 Epilithic diatoms from the Trout Beck, North Pennines: 1997-2011

R.W. Battarbee, E. M. Shilland & H.Yang

April 2012

### Epilithic diatoms from the Trout Beck, North Pennines: 1997-2011

Report to the Environmental Change Network

ECRC Research Report # 150

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#### April 2012

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ISSN: 1366-7300

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## **1 SITE DESCRIPTION**

"The Trout Beck is a headwater stream of the River Tees which drains Great Dun Fell, Hard Hill and Knock Fell in the North Pennines (Figure 1). The ECN sampling point (Grid Ref NY758335) is at 535m altitude and the catchment above this covers 1146 ha, rising to 848 m altitude (Figure 2). The geology is alternating strata of Carboniferous limestones, sandstone and shales. Blanket peat covers 90% of the catchment with skeletal soils towards the fell tops and small areas of limestone soils and alluvial soils. Vegetation is dominated by ling heather (*Calluna vulgaris*), cotton grass (*Eriophorum spp*) and *Sphagnum* moss. The catchment lies in Moor House National Nature Reserve which is owned by Natural England. Discharge is measured at a Compound Crump Gauging Station operated by the Environment Agency. The pH of Trout Beck averages 6.2 although there are wide fluctuations associated with the discharge. The site has a long history of ecological research." (<u>ECN website</u>, 2012).

## **2 INTRODUCTION**

Since 1997 the ECRC has received nine epilithic diatom samples per year from the Trout Beck, the principal stream draining the Moor House NNR and ECN site. The samples were taken and continue to be taken on three occasions each year (Spring, Summer and Autumn) from three close-by but different sampling stations in the stream (Figure 3):

- Location S01: just below the confluence with Netherhearth Sike
- Location S02: a few metres downstream just before the start of the solid rock stream bed
- Location S03: a bar of loose stones across the solid rock stream bed

The analyses presented in this report are for the full suite of samples taken from 1997 until 2011 (Appendix 3) with the exception of two missing samples as follows:

- Location S01 on 31/03/2004
- Location S01 on 26/09/2007

Diatoms were collected from the Trout Beck ECN site and prepared for analysis following protocols described in the United Kingdom Environmental Change Network's "Protocols for Standard Measurements at Freshwater Sites" (Sykes *et al.,* 1999).

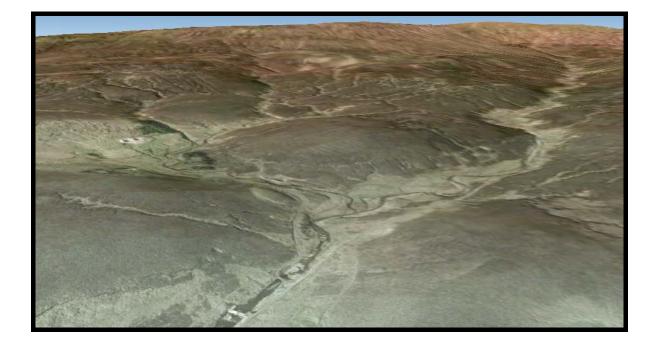
A sub-set of 17 samples (SO2 samples from April 1997 to October 2001) was analysed earlier by Annette Kreiser (Kreiser *et al.* 2002). Another set of 88 samples was analysed by Rick Battarbee (Battarbee & Shilland, 2010), which completed the time-series to 2008. This report adds 27 more recent samples taken between 2009 and 2011, with diatom analysis performed by Hong Yang.

Comparisons of counts by Kreiser, Battarbee and Yang showed no obvious taxonomic inconsistencies. Diatoms were examined at 1200 x magnification using a Leitz SM-Lux compound microscope (Kreiser), at 1000 x using a Leitz Orthoplan microscope (Battarbee) and at 1000 x magnification using a Nikon Eclipse E400 microscope (Yang), with phase contrast illumination utilised throughout. A minimum of 300 valves were identified and counted for each slide by Kreiser and Battarbee, whereas Yang counted between 500 and 600 valves. Some taxonomic difficulties were encountered, mainly in differentiating between *Gomphonema* taxa when observed in girdle view. Taxa are coded according to the ECRC diatom database coding system DIATCODE and a full list of taxa observed is shown in Appendix 1.



#### Figure 1 Trout Beck Sampling Area

Figure 2 Trout Beck Catchment



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Figure 3 Trout Beck Sampling Locations

## **3 DATA ANALYSIS AND PRESENTATION**

Data are held on a central Access database at the Environmental Change Research Centre (ECRC). In this report we present the primary data with graphs and summary statistics. Diatom diagrams show percentage abundances of individual species for each year of sampling or for each individual sample point. Only species occurring with a minimum abundance of 1.5% are presented. We also present a series of diversity indices including:

- Hill's N1 that approximates to the number of abundant species (Hill, 1973).
- Hill's N2 that approximates to the number of very abundant species in the sample.
- **Hill's E5** that is a measure of the evenness of species occurrences in a sample. E5 approaches zero as a single species becomes more dominant in the assemblage.

Multivariate statistical methods were applied to the epilithic diatom data. The linear method of Principal Components Analysis (PCA) was selected. PCA is an indirect gradient approach that provides a sensitive measure of between sample variance in the species assemblage. The analysis was performed using the program C2 (Juggins, 2007).

All new diatom data presented in this report have been formatted as stipulated by ECN protocols (Sykes *et al.*, 1999) and sent to the central ECN database administrator.

## 4 OBJECTIVES

The objectives of the analyses reported previously (Battarbee & Shilland 2010) were:

- to identify the diatoms present in the samples and to compare the composition of the assemblages between replicate stations, between seasons within a year, between the same season between years, and between years over time;
- to identify any evidence for long-term trends in species composition and overall biodiversity over time; and
- to examine the relationship between count size and species richness to assess the optimum count size for stream biodiversity studies.

Our objectives here remain the same, including the longer term aim of relating variation in diatom composition over time to environmental factors, including stream water chemistry and hydrology. Data for the additional three years presented here should enhance that aim.

## 5 RESULTS

### 5.1 Floristic change

The diatom data are shown in Figures 4-10. Figures 11-17 show PCA plots for species and sample scores. The diagrams (Figures 4-10) are identical to those in Battarbee & Shilland (2010) but with data from 2009, 2010 and 2011 added.

As described in Battarbee & Shilland (2010) Figure 4 shows the pattern of seasonal (spring, summer and autumn) and inter-annual variability in diatom composition, now over the 15 year period from 1997-2011. One species, *Achnanthes minutissima*, is dominant in all seasons and all years, varying from approximately 30 to 80% of the total count. There are two occasions in the time series when its relative abundance declines, first in 1998-1999 and second in 2004-2005. In both cases there are corresponding increases in the relative

abundance of *Gomphonema* taxa, especially *G. pumilum*. The reason for this reciprocal variability is unknown as the taxa concerned have similar lifeforms and similar water quality requirements. Moreover, although the relative abundance of all *Gomphonema* taxa increase as *A. minutissima* decreases the different *Gomphonema* species also behave independently (cf.Figures 11-17), suggesting that there are fine controls on the competitive success of individual taxa within the *Gomphonema* genus. The additional three years data conform to the standard pattern for the site of dominance by *A. minutissima*. They highlight further the periods of deviation from this pattern, and reinforce the need for an explanation especially with respect to the influence of hydrological events potentially leading to temporary changes in macroinvertebrate grazing pressure.

The similarities between the assemblages at the beginning and end of the record show that no significant longer term changes in environmental conditions have occurred in the stream over the last 15 years.

Figures 5-7 show data for the three sample points, SO1, SO2, SO3, illustrating differences between epilithic assemblages within the ~50 m reach of stream sampled (cf. Figure 3). Whilst there is considerable variability between samples all three sites show the same temporal pattern as described above. They are also structured in a very similar way (cf. Figures 12-14), suggesting that there are no significant micro-scale differences in habitat or environmental conditions between the sample points. The data for the additional years conform to the patterns previously observed.

Figures 8-10 present the data by season: spring (Figure 8), summer (Figure 9) and autumn (Figure 10). Again the relative abundances of the different taxa between seasons are broadly the same, showing no evidence for a regular seasonal succession in floristic composition. *A. minutissima* dominates in all samples and all seasons and *Gomphonema* taxa show the same inter-annual patterns irrespective of season. The single, clear outlier is autumn 2002 where all three samples show relatively low abundances of *A. minutissima* and relatively high abundances of *Fragilaria capucina* var. *gracilis, Synedra ulna* and *Diatoma tenue* var. *elongatum.* These samples and species are clearly differentiated in the PCAs (Figures 11 and 17). Again the reason for these differences is unknown, but the between-sample consistency of this assemblage suggests that environmental conditions in the autumn of 2002 were different from both previous and following years. Examination of hydrochemical conditions over this period of time might provide an explanation. The additional data show no new seasonal differences or a recurrence of the switch in species dominance previously seen in autumn 2002.

### 5.2 Diversity

Figure 18 shows the number of taxa encountered for: (i) each sample (combined field sample from five stones) after a count of 300 valves per sample (SO1, SO2, and SO3) in 1997-2008 and 500 valves per samples in 2009-2011; (ii) for the site as a whole for any one season (SO1, SO2, and SO3 combined) giving a count size of 900 valves (1997-2008) or 1500 valves (2009-2011) and; (iii) for the site as a whole for any one year, by combining three samples and three seasons to

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generate a count size of 2,700 (1997-2008) or 4,500 (2009-2011). The mean number of taxa observed for these three sample groups were 15.3, 23.9 and 37.4 in the samples where 300 valves were counted (1997-2008), 18.5, 26.3 and 36 in the samples where 500 valves were counted (2009-2011) and an overall average for the whole time-series of 16.0, 24.6 and 37.5 respectively. This compares with the ca. 100 taxa encountered overall across the 15 year data-set (Figure 26, Appendix 2) representing a total combined count of 48,331 diatom valves. As there has been little or no overall change in environmental conditions over the last 15 years, indicated by the stability of the diatom assemblage and the addition of no new taxa in the 2009-11 data, it is probable that this represents the total diatom species pool in the Trout Beck at any one time.

Figures 19–25 show Hill's N1, Hill's N2 and E5 as additional measures of diversity. Figure 19 shows the pattern of seasonal (spring, summer and autumn) and interannual variability in diatom species diversity over the 15 year sample period, averaged for all three sampling locations.

Figures 20-22 show diversity data for the three sample points, SO1, SO2, SO3, illustrating differences between epilithic assemblages within the reach of stream sampled (cf. Figure 2) and Figures 23-25 present the data averaged over the three sampling locations by season: spring (Figure 23), summer (Figure 24) and autumn (Figure 25).

The data show relatively little change over time, although there is a peak in Hill's N1 and N2 for the Summer and Autumn samples of 1998, especially at sampling points S01 and S03 (Figures 20, 22, 24 and 25), and there is a general increase over time in Hill's N1 and N2 in spring samples (Figure 23).

## 6 SUMMARY AND CONCLUSIONS

Analysis of the epilithic diatom samples from the Trout Beck now covers 15 years from 1997 to 2011. The flora remains relatively stable, dominated by *Achnanthes minutissima*, an abundant and globally widespread benthic diatom found in both lakes and streams of circumneutral pH. It is a diatom that is very sensitive to acidification, very rarely found in waters with pH < 5.5, and an indication here that the Trout Beck has retained a relatively high acid neutralising capacity over recent decades despite its location in a high acid deposition area in the UK. The addition of three more years of data confirms the pattern observed previously (Battarbee & Shilland 2010).

The dominance of *A. minutissima* is somewhat reduced both in the early part of the time-series and in 2004-2005 when the abundance of *Gomphonema* taxa increase reciprocally. It is also much less common in the autumn samples of 2002, when it is replaced by *Fragilaria capucina* var. *gracilis*, *Synedra ulna* and *Diatoma tenue* var. *elongatum*.

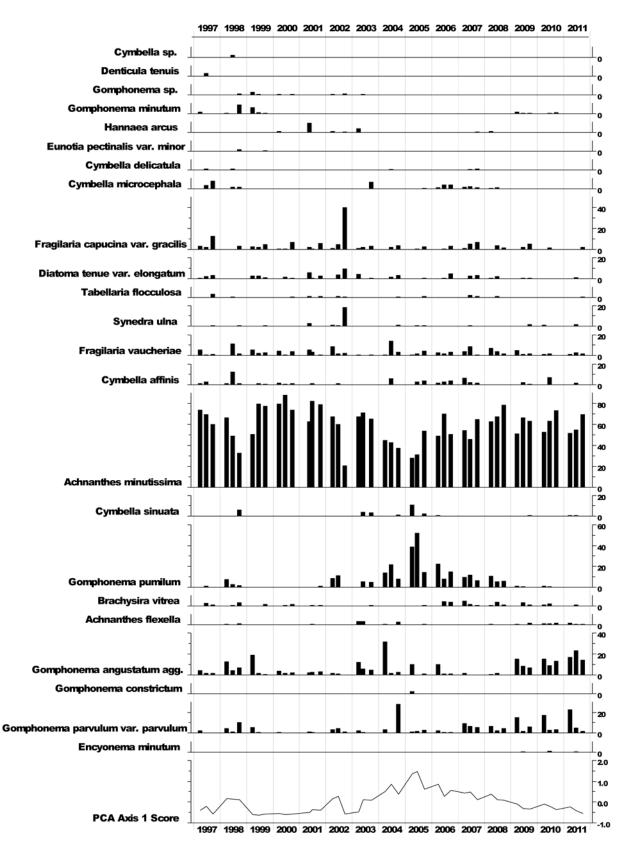
The influence of these different taxa on the structure of the assemblages is seen clearly in the PCAs (Figs. 11-17).

As these floristic switches occur quite abruptly, either inter-annually or seasonally, and as there is little evidence from their pH affinity that the species changes are caused by acid episodes (that might be expected at more acid sensitive sites), it is more probable that the occasional depressions in the abundance of *A. minutissima* are related to physical factors such as changes in discharge and consequent alterations to the physical habitat of the stream bed, rather than to changes in water chemistry. As *A. minutissima* is a diatom well-adapted to invertebrate grazing pressure its temporary decrease may be related to a reduction in grazing pressure caused by high discharge events. Its replacement by long-stalked taxa, such as *Gomphonema* or needle-shaped taxa such as *Synedra acus* accords with this conjecture.

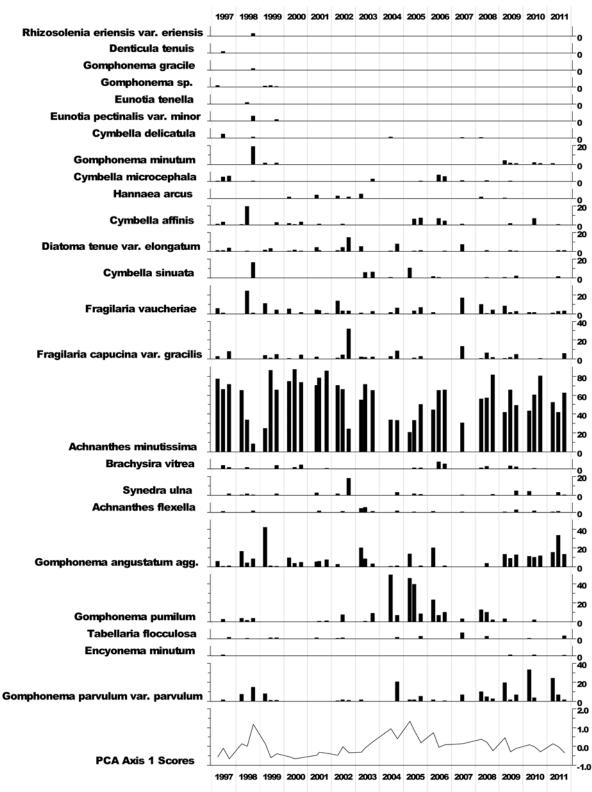
There is little overall change in diatom diversity over the time period monitored. Species richness is similar to that of other epilithic stream habitats in the UK uplands. Typically 15 taxa are encountered in a standard count of 300 diatom valves, and 18 species in a count of 500 valves, from a total species pool of approximately 100.

Combined analysis of the diatom data and environmental data (especially for chemistry, flow and grazing pressure) is required to explain the floristic changes identified.

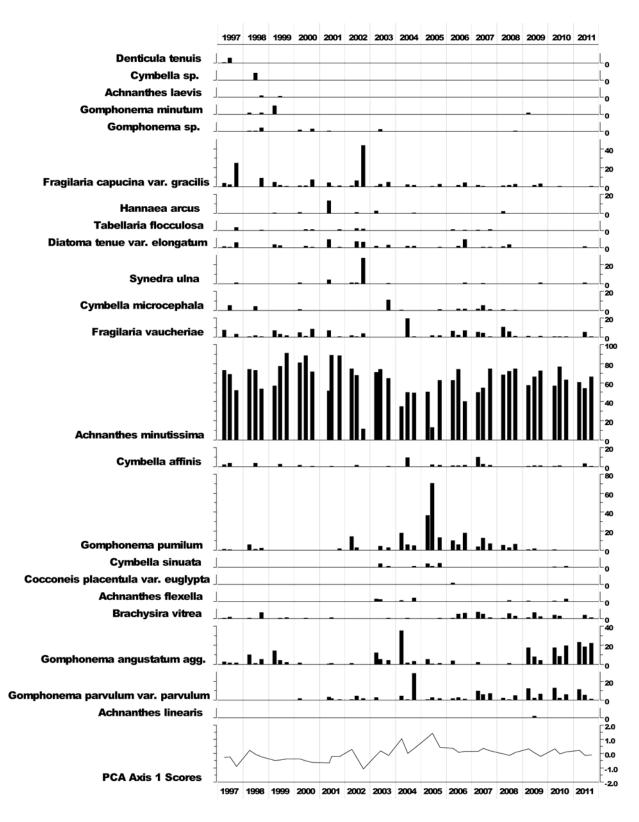




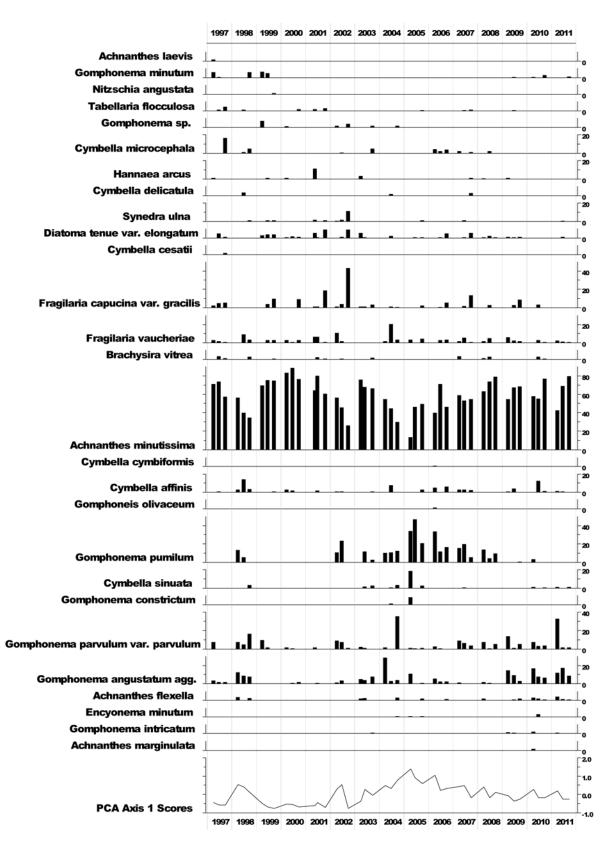
# Figure 5 Trout Beck Diatom Percentage Abundances from Sampling Point S01



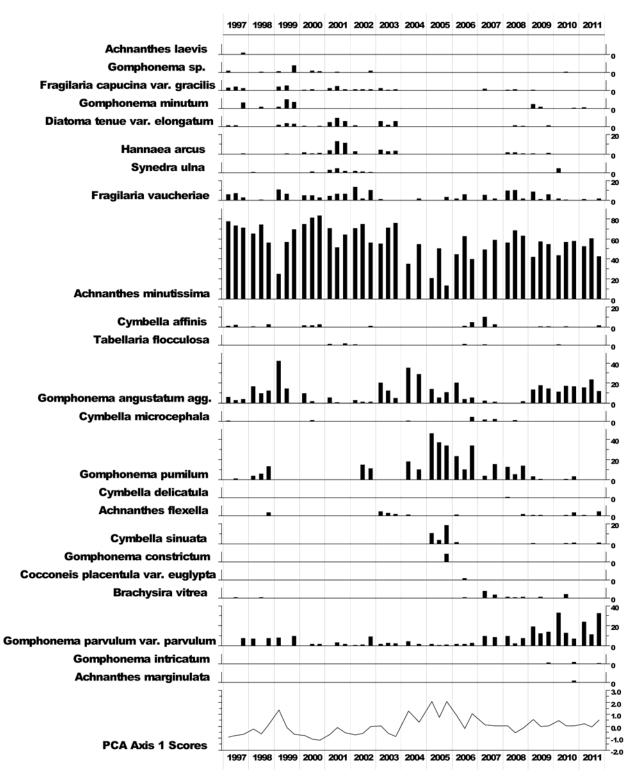
# Figure 6 Trout Beck Diatom Percentage Abundances from Sampling Point S02



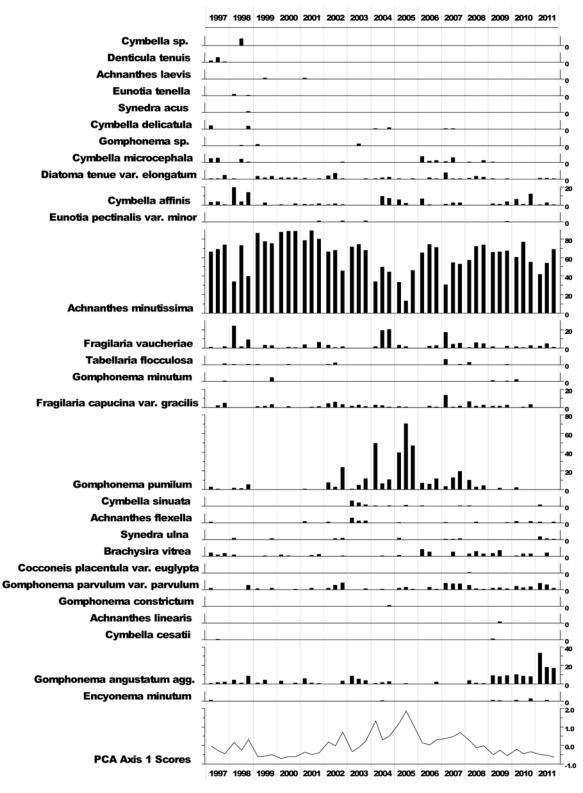
# Figure 7 Trout Beck Diatom Percentage Abundances from Sampling Point S03



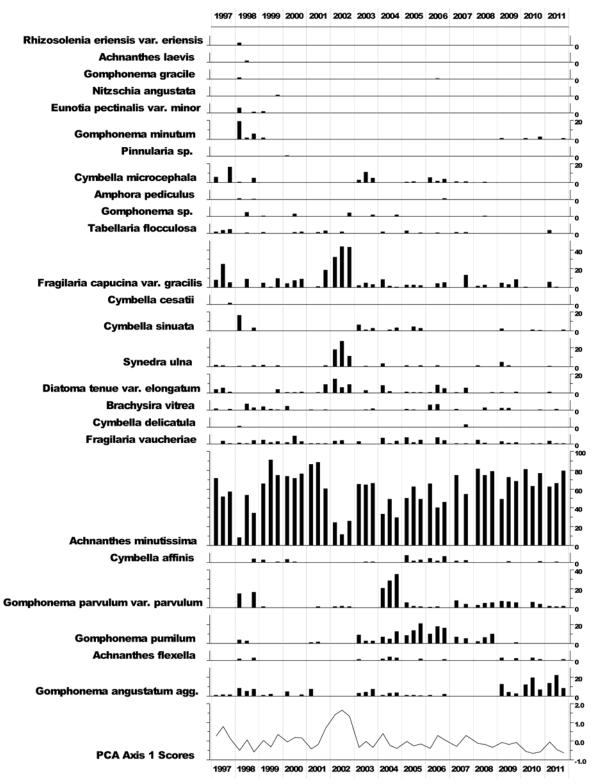
# Figure 8 Trout Beck Diatom Percentage Abundances from S01, S02 and S03 Spring Samples



## Figure 9 Trout Beck Diatom Percentage Abundances from S01, S02 and S03 Summer Samples



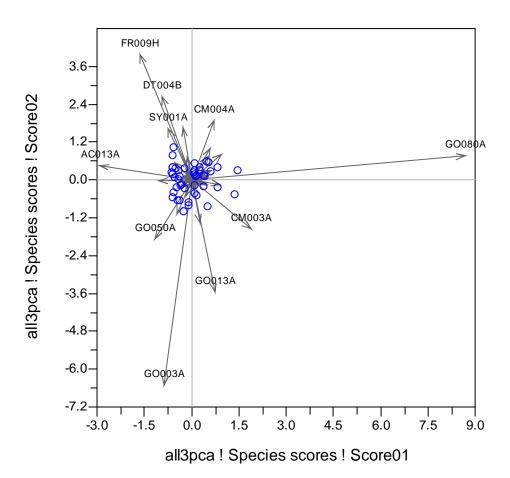
## Figure 10 Trout Beck Diatom Percentage Abundances from S01, S02 and S03 Autumn Samples



#### **Table 1 Diatom PCA Statistics**

λ <sup>ρςα</sup>	Axis 1 Eigenvalue	Axis 2 Eigenvalue	Axis 3 Eigenvalue	Axis 4 Eigenvalue
All 3 samples	0.27	0.21	0.12	0.07
S01 samples	0.23	0.20	0.15	0.08
S02 samples	0.22	0.21	0.13	0.08
S03 samples	0.28	0.18	0.09	0.08
Spring samples	0.57	0.22	0.11	0.05
Summer samples	0.34	0.15	0.11	0.08
Autumn samples	0.26	0.21	0.14	0.08

Figure 11 Trout Beck Diatom PCA Species and Sample Scores: All 3 samples



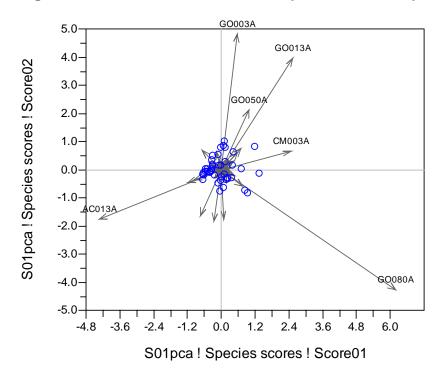
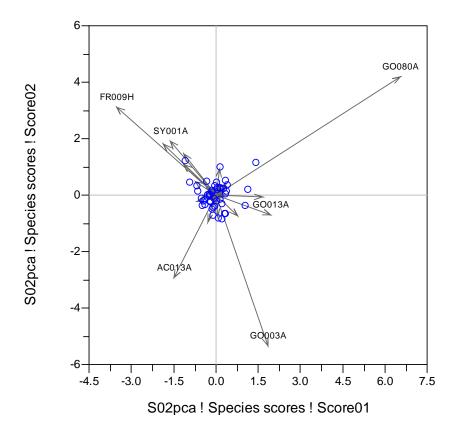


Figure 12 Trout Beck Diatom PCA Species and Sample Scores: S01 samples

Figure 13 Trout Beck Diatom PCA Species and Sample Scores: S02 samples



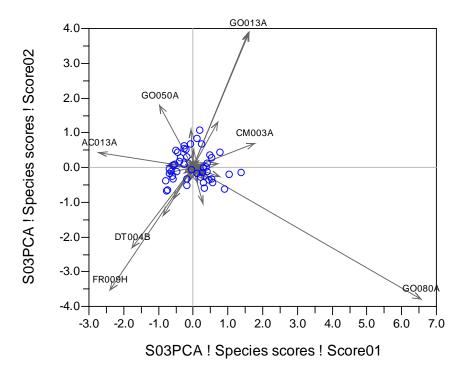
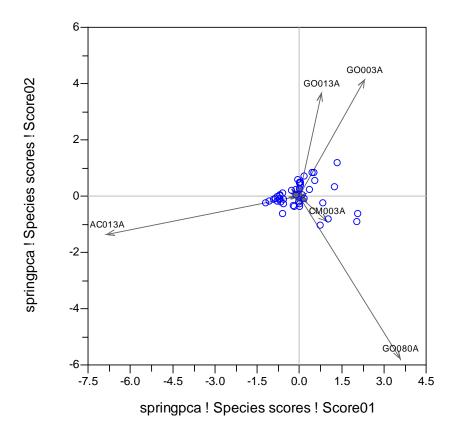


Figure 14 Trout Beck Diatom PCA Species and Sample Scores: S03 samples







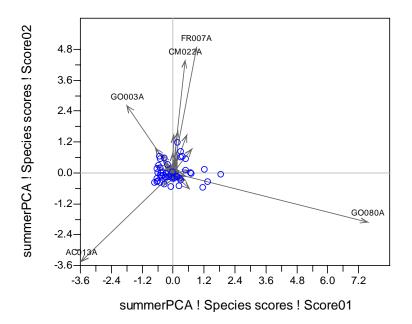
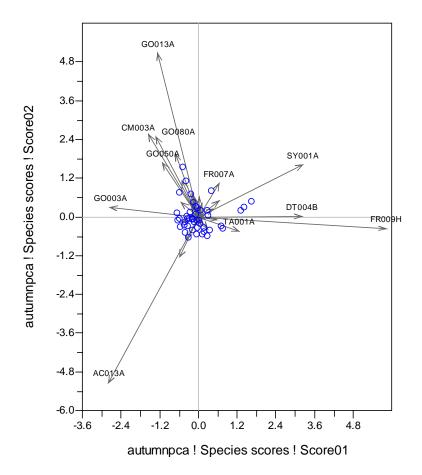
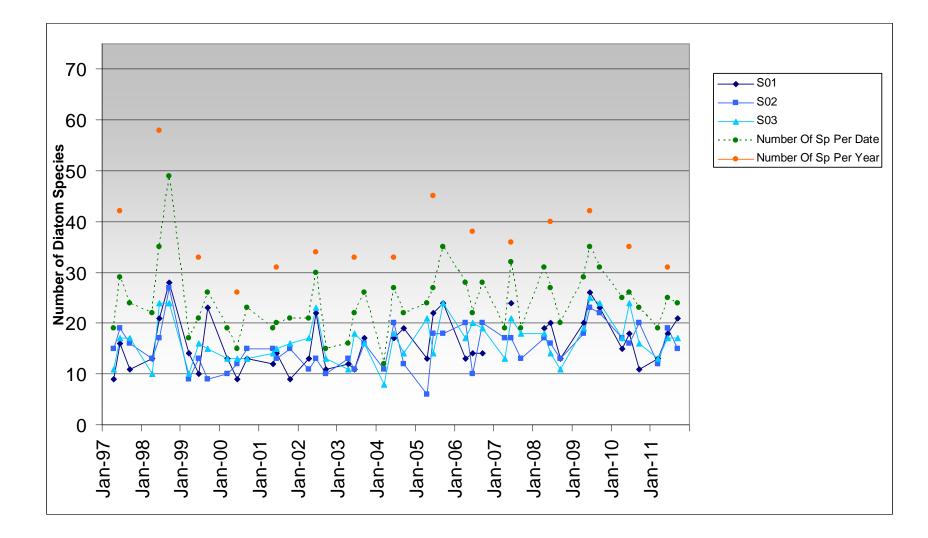


Figure 17 Trout Beck Diatom PCA Species and Sample Scores: Autumn samples





#### Figure 18 Number of Diatom Species per Sample, Date, Year and Overall Total

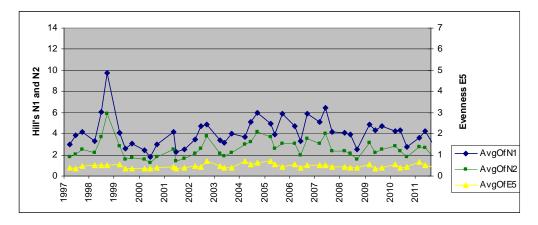


Figure 19 Trout Beck Diatom Diversity Statistics Mean of Points S01, S02 and S03

Figure 20 Trout Beck Diatom Diversity Statistics from Sampling Point S01

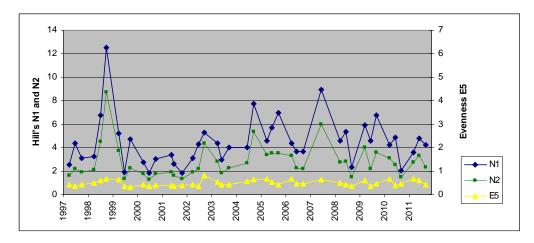
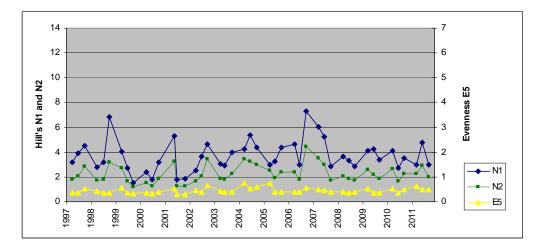


Figure 21 Trout Beck Diatom Diversity Statistics from Sampling Point S02



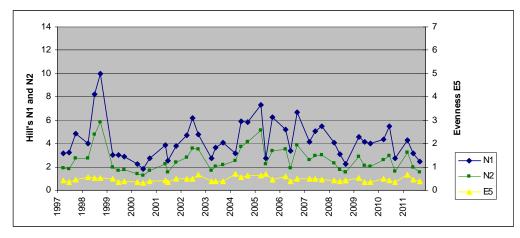


Figure 22 Trout Beck Diatom Diversity Statistics from Sampling Point S03

Figure 23 Trout Beck Diatom Diversity Statistics Mean of Points S01, S02 and S03: Spring Samples

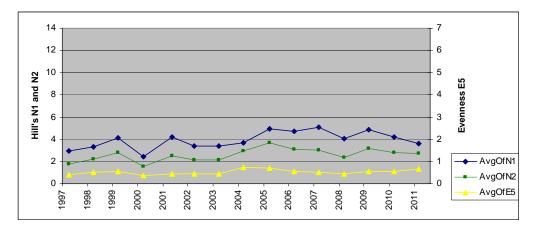
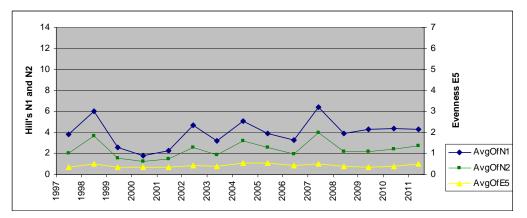


Figure 24 Trout Beck Diatom Diversity Statistics Mean of Points S01, S02 and S03: Summer Samples



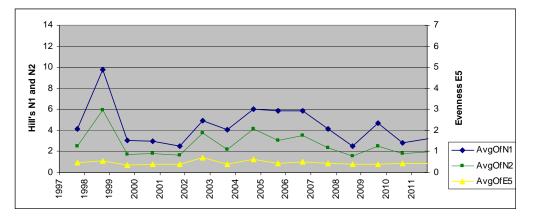


Figure 25 Trout Beck Diatom Diversity Statistics Mean of Points S01, S02 and S03: Autumn Samples

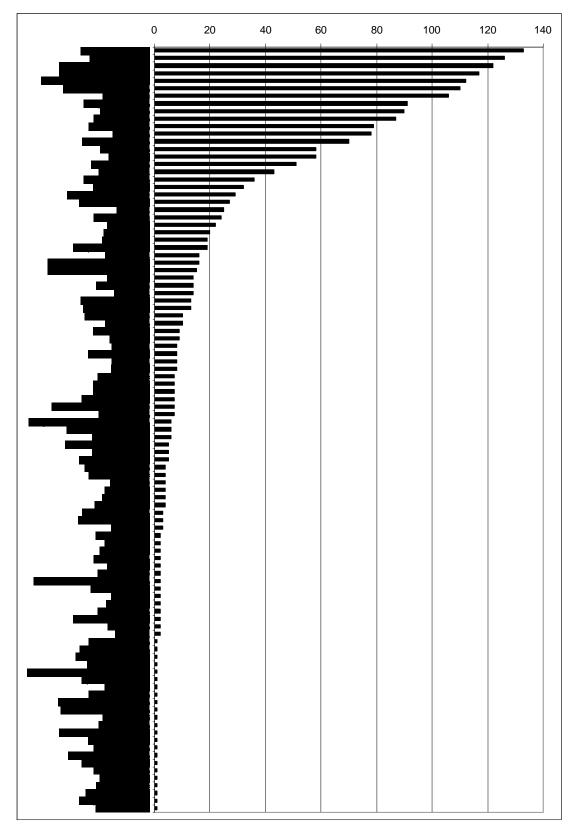


Figure 26 Diatom Species Ordered by Number of Occurrences (N<sub>max</sub> 133)

## 7 ACKNOWLEDGEMENTS

Funding for this work was provided by the Environmental Change Network. Bev Dodd undertook much of the field sampling and Kevin Roe prepared the diatom slides. Seventeen samples were counted by Annette Kreiser (Kreiser *et al.* 2002) and 88 by Rick Battarbee (Battarbee & Shilland, 2010) under earlier contracts.

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## 9 APPENDICES

### Appendix 1 Trout Beck Diatom Species and ECRC Database DIATCODE

Taxon	Taxon Code
Achnanthes flexella	AC025A
Achnanthes helvetica	AC134A
Achnanthes laevis	AC083A
Achnanthes lanceolata	AC001A
Achnanthes laterostrata	AC018A
Achnanthes linearis	AC002A
Achnanthes marginulata	AC022A
Achnanthes minutissima	AC013A
Achnanthes pseudoswazi	AC004A
Achnanthes pusilla	AC035A
Achnanthes sp.	AC9999
Amphora pediculus	AM012A
Aulacoseira sp.	AU9999
Brachysira vitrea	BR001A
Cocconeis pediculus	CO005A
Cocconeis placentula	CO001A
Cocconeis placentula var. euglypta	CO001B
Cyclotella meneghiniana var. meneghiniana	CY003A
Cymbella affinis	CM022A
Cymbella amphicephala	CM016A
Cymbella cesatii	CM015A
Cymbella cymbiformis	CM007A
Cymbella delicatula	CM038A
Cymbella descripta	CM052A
Cymbella gracilis	CM018A
Cymbella laevis	CM012A
Cymbella microcephala	CM004A
Cymbella sinuata	CM003A
Cymbella sp.	CM9999
Denticula tenuis	DE001A
Diatoma tenue var. elongatum	DT004B
Encyonema hebridicum	EY003A
Encyonema minutum	EY011A
Encyonema turgidum	EY018A
Eunotia arcus	EU013A
Eunotia curvata	EU049A
Eunotia curvata var. subarcuata	EU049B
Eunotia denticulata	EU015A
Eunotia exigua	EU009A
Eunotia incisa	EU047A
Eunotia Iunaris	EU006A

Taxon	Taxon Code
Eunotia lunaris var. subarcuata	EU006B
Eunotia meisteri	EU020A
Eunotia paludosa	EU040A
Eunotia pectinalis	EU002A
Eunotia pectinalis var. minor	EU002B
Eunotia pectinalis var. minor f. impressa	EU002E
Eunotia pectinalis var. ventralis	EU002C
Eunotia rhomboidea	EU011A
Eunotia sp.	EU9999
Eunotia tenella	EU004A
Fragilaria capucina var. gracilis	FR009H
Fragilaria vaucheriae	FR007A
Fragilaria virescens	FR005A
Fragilaria sp.	FR9999
Frustulia rhomboides var. saxonica	FU002B
Gomphoneis olivaceum	GM001A
Gomphonema acuminatum var. coronatum	GO006C
Gomphonema angustatum agg.	GO003A
Gomphonema constrictum	GO010A
Gomphonema gracile	GO004A
Gomphonema intricatum	GO014A
Gomphonema minutum	GO050A
Gomphonema parvulum var. parvulum	GO013A
Gomphonema pumilum	GO080A
Gomphonema sp.	GO9999
Hannaea arcus	HN001A
Krasskella kriegerana	KR001A
Meridion circulare	MR001A
Navicula cocconeiformis	NA032A
Navicula cryptocephala	NA007A
Navicula gracilis	NA029A
Navicula lanceolata	NA009A
Navicula radiosa var. radiosa	NA003A
Navicula radiosa var. tenella	NA003B
Navicula rhyncocephala	NA008A
Navicula sp.	NA9999
Navicula viridula var. viridula	NA027A
Nitzschia angustata	NI020A
Nitzschia dissipata	NI015A
Nitzschia fonticola	NI002A
Nitzschia gracilis	NI017A
Nitzschia palea	NI009A
Nitzschia perminuta	NI005A
-	NI025A
Nitzschia recta	1
	NI9999
Nitzschia recta Nitzschia sp. Pinnularia appendiculata	NI9999 PI014A

Taxon	Taxon Code
Pinnularia irrorata	PI023A
Pinnularia sp.	PI9999
Pinnularia subcapitata	PI022A
Rhizosolenia eriensis var. eriensis	RZ011A
Rhoicosphenia abbreviata	RC002A
Sellaphora rectangularis	SL007A
Surirella angustata	SU001A
Surirella ovata	SU002A
Synedra acus	SY003A
Synedra pulchella	SY008A
Synedra sp.	SY9999
Synedra ulna	SY001A
Tabellaria flocculosa	TA001A

## Appendix 2 Trout Beck Diatom Species in Order of Number of Occurrences

Taxon	Taxon Code	Number Of Occurrences
Achnanthes minutissima	AC013A	133
Fragilaria vaucheriae	FR007A	126
Gomphonema angustatum agg.	GO003A	122
Fragilaria capucina var. gracilis	FR009H	117
Gomphonema parvulum var. parvulum	GO013A	112
Diatoma tenue var. elongatum	DT004B	110
Cymbella affinis	CM022A	106
Gomphonema pumilum	GO080A	91
Brachysira vitrea	BR001A	90
Achnanthes flexella	AC025A	87
Tabellaria flocculosa	TA001A	79
Synedra ulna	SY001A	78
Cymbella microcephala	CM004A	70
Cymbella sinuata	CM003A	58
Hannaea arcus	HN001A	58
Encyonema minutum	EY011A	51
Gomphonema sp.	GO9999	43
Gomphonema minutum	GO050A	36
Cymbella delicatula	CM038A	32
Eunotia pectinalis var. minor	EU002B	29
Gomphonema intricatum	GO014A	27
Eunotia sp.	EU9999	25
Achnanthes linearis	AC002A	24
Eunotia tenella	EU004A	22
Denticula tenuis	DE001A	20
Cymbella cesatii	CM015A	19
Gomphonema constrictum	GO010A	19
Achnanthes sp.	AC9999	16
Cocconeis placentula var. euglypta	CO001B	16
Frustulia rhomboides var. saxonica	FU002B	15

Taxon	Taxon Code	Number Of Occurrences
Eunotia exigua	EU009A	14
Meridion circulare	MR001A	14
Navicula sp.	NA9999	14
Achnanthes marginulata	AC022A	13
Encyonema hebridicum	EY003A	13
Cymbella cymbiformis	CM007A	10
Nitzschia palea	NI009A	10
Amphora pediculus	AM012A	9
Eunotia incisa	EU047A	9
Cymbella sp.	CM9999	8
Gomphonema gracile	GO004A	8
Nitzschia sp.	NI9999	8
Synedra acus	SY003A	8
Achnanthes laevis	AC083A	7
Cymbella descripta	CM052A	7
Fragilaria virescens	FR005A	7
Navicula cryptocephala	NA007A	7
Rhizosolenia eriensis var. eriensis	RZ011A	7
Synedra pulchella	SY008A	7
Gomphonema acuminatum var. coronatum	GO006C	6
Navicula viridula var. viridula	NA027A	6
Nitzschia angustata	NI020A	6
Navicula radiosa var. radiosa	NA003A	5
Nitzschia perminuta	NI005A	5
Pinnularia appendiculata	PI014A	5
Achnanthes lanceolata	AC001A	4
Cocconeis pediculus	CO005A	4
Eunotia arcus	EU013A	4
Eunotia curvata	EU049A	4
Navicula gracilis	NA029A	4
Nitzschia fonticola	NI002A	4
Gomphoneis olivaceum	GM001A	3
Navicula cocconeiformis	NA032A	3
Pinnularia sp.	PI9999	3
Achnanthes pusilla	AC035A	2
Aulacoseira sp.	AU9999	2
Cymbella gracilis	CM018A	2
Eunotia denticulata	EU015A	2
Eunotia lunaris	EU006A	2
Eunotia pectinalis	EU002A	2
Eunotia pectinalis var. minor f. impressa	EU002E	2
Eunotia rhomboidea	EU011A	2
Fragilaria sp.	FR9999	2
Nitzschia recta	NI025A	2
Pinnularia irrorata	PI023A	2
Rhoicosphenia abbreviata	RC002A	2
Surirella ovata	SU002A	2

Taxon	Taxon Code	Number Of Occurrences
Synedra sp.	SY9999	2
Achnanthes helvetica	AC134A	1
Achnanthes laterostrata	AC018A	1
Achnanthes pseudoswazi	AC004A	1
Cocconeis placentula	CO001A	1
Cyclotella meneghiniana var. meneghiniana	CY003A	1
Cymbella amphicephala	CM016A	1
Cymbella laevis	CM012A	1
Encyonema turgidum	EY018A	1
Eunotia curvata var. subarcuata	EU049B	1
Eunotia lunaris var. subarcuata	EU006B	1
Eunotia meisteri	EU020A	1
Eunotia paludosa	EU040A	1
Eunotia pectinalis var. ventralis	EU002C	1
Krasskella kriegerana	KR001A	1
Navicula lanceolata	NA009A	1
Navicula radiosa var. tenella	NA003B	1
Navicula rhyncocephala	NA008A	1
Nitzschia dissipata	NI015A	1
Nitzschia gracilis	NI017A	1
Pinnularia borealis	PI012A	1
Pinnularia subcapitata	PI022A	1
Sellaphora rectangularis	SL007A	1
Surirella angustata	SU001A	1

### Appendix 3 Diatom sampling dates

Sampling date	Number of Samples
02/04/1997	3
24/06/1997	3
24/09/1997	3
01/04/1998	3
30/06/1998	3
23/09/1998	3
31/03/1999	3
22/06/1999	3
22/09/1999	3
29/03/2000	3
20/06/2000	3
27/09/2000	3
23/05/2001	3
26/06/2001	3
24/10/2001	3

Sampling date	Number of Samples
10/04/2002	3
25/06/2002	3
25/09/2002	3
02/04/2003	3
01/06/2003	3
24/09/2003	3
31/03/2004	2
23/06/2004	3
29/09/2004	3
12/04/2005	3
15/06/2005	3
28/09/2005	3
05/04/2006	3
28/06/2006	3
27/09/2006	3
04/04/2007	3
20/06/2007	3
26/09/2007	2
02/04/2008	3
25/06/2008	3
24/09/2008	3
01/04/2009	3
24/06/2009	3
23/09/2009	3
07/04/2010	3
23/06/2010	3
22/09/2010	3
30/03/2011	3
22/06/2011	3
22/09/2011	3