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**A Rationale for the Use of Artificial Substrata to Enhance  
Diatom-Based Monitoring of Eutrophication in Lowland  
Rivers.**

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# A rationale for the use of artificial substrata to enhance diatom-based monitoring of eutrophication in lowland rivers

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## Abstract

With the introduction of new European directives governing water quality, the need to monitor eutrophication in rivers has been recognized. The inclusion of a reliable biotic component in any such monitoring scheme is considered necessary. Diatoms have been shown to display a direct response to trophic status and are therefore being used in preliminary work in this field. The sampling of river diatoms has relied on the river site having clean cobbles, or other solid substrata, from which to collect the epilithon. From a total of 58 lowland river sites visited only 31 had a good epilithic community. The need therefore exists for a more reliable sampling method in these systems. Artificial substrata have a number of reported advantages over the natural communities: lower sample variability, consistency between sites, placement at the point of interest and ease of use. Data are presented here to compare three different types of artificial substrata (rough tile, smooth tile and rope) with the natural communities (epilithon epipelton and epiphyton) at two eutrophic river sites. Within sample variation is shown to be lower on the artificial substrata while a high diversity is maintained on the rope and rough tiles. Further advantages of using artificial substrata are described and the logistics of their applied use are discussed.

## Introduction

In the past the focus for British river water quality has been almost entirely on organic pollution and the use of macroinvertebrates and simple physico-chemical method for monitoring has proved adequate. In the light of the new European legislation, however, the need has arisen to monitor more subtle constituents of our rivers, in particular nitrogen and phosphorus. Traditional methods of biological assessment are not sensitive to trophic status and thus there is a need to develop a reliable biological system to monitor eutrophication in rivers. Due to the nature of N and P pollution the use of photosynthetic organisms has proved the most effective means of assessment (Whitton & Kelly 1995).

The use of diatoms to monitor river water quality has been well documented, mainly within mainland Europe (Descy 1979, Whitton *et al.* 1991), but the focus has been on organic pollution and not eutrophication. It is only in the last few years that a diatom-based trophic index, the TDI (Kelly & Whitton 1995), was developed to fulfill the needs of the water regulators. Diatoms have proved an ideal group to use in the monitoring of ambient water quality, including eutrophication, for a number of reasons:

- Diatoms are found universally within aquatic habitats; colonizing almost all submerged surfaces.
- They are very sensitive to changes in water quality and their ecology is well understood.
- The rapid cell cycle of diatoms means that they react quickly to any change in water quality.
- The collection of diatom samples is fast and easily standardized between different collectors.
- The preparation of permanent slides allows for long term sample storage and good taxonomic quality control.

The sampling of river diatoms for water quality assessment has been based almost entirely on the natural epilithic community (Round 1993). The careful scraping or brushing of a set number of cobbles has been recommended. When applied to the slower flowing reaches of lowland rivers, however, this habitat is often either missing or, where cobbles do occur, they are covered by a layer of sediment thus attributing to considerable variation between samples. A reliable diatom-based monitoring system therefore needs to incorporate between site control of the sampled substratum to ensure observed changes in the diatom flora are due to water quality and not

habitat differences. In lowland rivers the only way to achieve this is by the use of introduced artificial substrata.

Artificial substrata have been used extensively in the study of diatom ecology, in both rivers and lakes, and have shown to provide a number of advantages:

- A reduction in variation between replicate samples.
- Placement at the point of interest, e.g. up stream and down stream of an effluent discharge.
- They ensure substratum consistency is maintained between sites.
- Exposure time can be controlled allowing the study of equal age communities and also preventing a build up of dead, possibly allochthonous, cells.
- Artificial substrata are simple to sample quantitatively.

The aim of this study was to identify a suitable artificial substratum which would support a representative diatom flora while also being simple to use with a high rate of recovery. The latter point is important for the applied use of an artificial substratum; loss from the site would result in increased expense and loss of data.

### Study Sites

Two sites (Elst U/S & Elst D/S) were investigated on the River Wey near Elstead (Surrey). Upstream of Elstead the River Wey receives high levels of sewage effluent from Alton (Hampshire) and Farnham which are both greater than 10,000 population equivalent sewage treatment works (STW). As well as these large inputs there are also a number of smaller STWs discharging into the river. The Elstead sites are therefore relatively nutrient rich, with filterable reactive phosphorus levels FRP in the region of  $1000 \mu\text{g l}^{-1}$  (fig. 1).

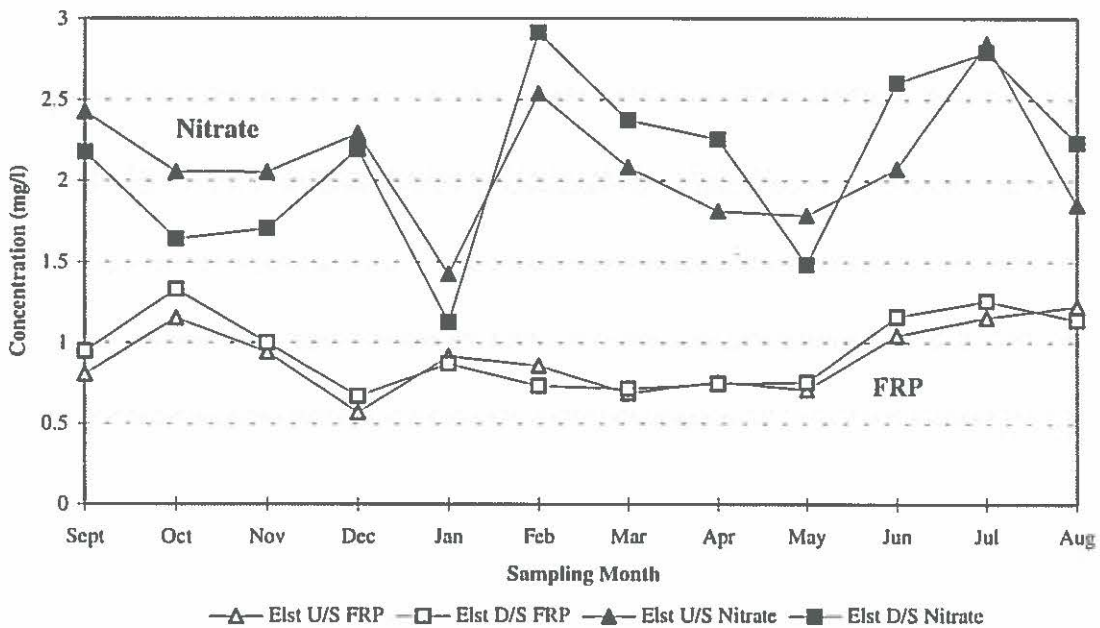


Figure 1. Annual FRP and nitrate at the two River Wey sites at Elstead

The two sites were 2 km apart and, although slightly different in physical character, chemically very similar. Elst U/S (NGR SU904437) was a slower flowing and deeper site and Elst D/S (NGR SU922439) was faster and shallow. Both sites had a total alkalinity of  $100 \text{ mg l}^{-1}$  (bicarbonate) and a total ionic strength of  $400 \mu\text{S cm}$ . For the purposes

of this study the sites will therefore be considered together unless otherwise stated.

Data on the logistics of using artificial substrata were collected from 58 sites within and around the Thames catchment.

## Methods

At both sites a range of different substrata, both natural and artificial, were investigated. The substrata under investigation are shown in table 1. Each substratum was sampled in triplicate to determine the amount of within site variation in diatom species. All the artificial substrata were put in place in mid September 1995 and left for 31 days. An exposure time of one month has been determined as sufficient by a number of workers (Stevenson & Lowe 1986) and has provided good diatom data in a similar river study (Author, unpublished).

Placement of the different artificial substrata has been subject to considerable investigation in a previous study (Author, unpublished). The tile samples were placed directly on to the river bed, without any form of attachment. Although this does make the possibility of loss during high flow conditions more likely, other methods tested had more serious problems associated with them. For example; if the tile was pegged to the river bed, the peg collected detritus which covered the whole tile; more elaborate frames, constructed to hold a tile to the river bed, often resulted in removal by the public, despite efforts made to label the frame or to conceal it. The rope samples were secured using a 30 cm stake hammered flush with the river bed. This method resulted in very few losses.

Substratum	Description & Collection
<i>Natural:</i>	
Sediment (epipelton)	A sample of fine surface sediment from which the live diatoms were extracted into lens tissue (Eaton & Moss 1966)
Submerged Macrophyte (epiphyton)	Approx 10 g (wet weight) from the tips of a submerged aquatic plant. This was kept constant to one species ( <i>Ranunculus</i> sp.)
Rock Scrape (epilithon)	One cobble of >20cm <sup>2</sup> . Selection of a cobble free of sediment and other algae was made. Diatoms removed by gentle brushing.
<i>Artificial:</i>	
Smooth tile	An unglazed floor tile, lacking in surface character. Placed directly on the river bed for 31 days. Sampled by delimiting a 25 cm <sup>2</sup> area and gently scrubbing with a toothbrush.
Rough tile	An unglazed roof tile with a highly textured surface. Placed directly on the river bed for 31 days. Sampled by delimiting a 25 cm <sup>2</sup> area and gently scrubbing with a toothbrush.
Rope	A 25 cm length of "cheap" polypropylene rope, staked to the river bed. Exposed for 31 days and sampled by removing the top 5 cm. In place it closely mimics a submerged Macrophyte in form.

Table 1. Substrata types

All the substrata were collected in mid October and the diatoms preserved in Lugol's iodine. Sub-samples of the tile and epilithic diatoms were taken for live analysis. All the samples were then prepared using standard techniques (Battarbee 1986). Rope samples were treated in a similar way to epiphyton samples. The 5 cm section of rope was heated in a 250 ml beaker with 50 ml of 30% hydrogen peroxide for 3 hours and then carefully removed. The remains of the rope were then back washed into the beaker with distilled water. Permanent slides, mounted in Naphrax™, were made for all samples. Diatoms were counted using phase contrast, oil immersion light microscopy at a magnification of x1250. Approximately 400 ± 50 diatom valves were counted on each slide. The sub-samples from the tile and epilithic diatoms were viewed uncleaned, at a magnification of x600, to assess numbers of living and dead cells at

time of sampling. Although it is very difficult to identify diatoms in the live state, the ratio of live:dead cells is easily assessed and can provide valuable information which is lost with traditional oxidative preparation techniques.

The diatom counts data were inputted into a specific diatom database, Amphora (Beare 1996), and down loaded in Cornell Condensed format for all further analysis. Detrended Correspondence Analysis (DCA) (Hill & Gauch) was used to compare sample similarity and Hill's N2 (Hill 1973) was used as a measure of species diversity. Ordinations and Hill's N2 diversities were calculated in Canoco (ter Braak 1988, 1990) and ordinations plotted in Calibrate (Juggins & ter Braak 1996). Information is also presented here to demonstrate the practicalities of using artificial substrata.

## Results

From the 36 samples (6 substratum types in triplicate from two sites) a total of 115 diatom taxa were identified. The examination of these data by

indirect gradient analysis (DCA) showed a high degree of similarity between the two sites but the between substratum variation was relatively high (fig. 2)

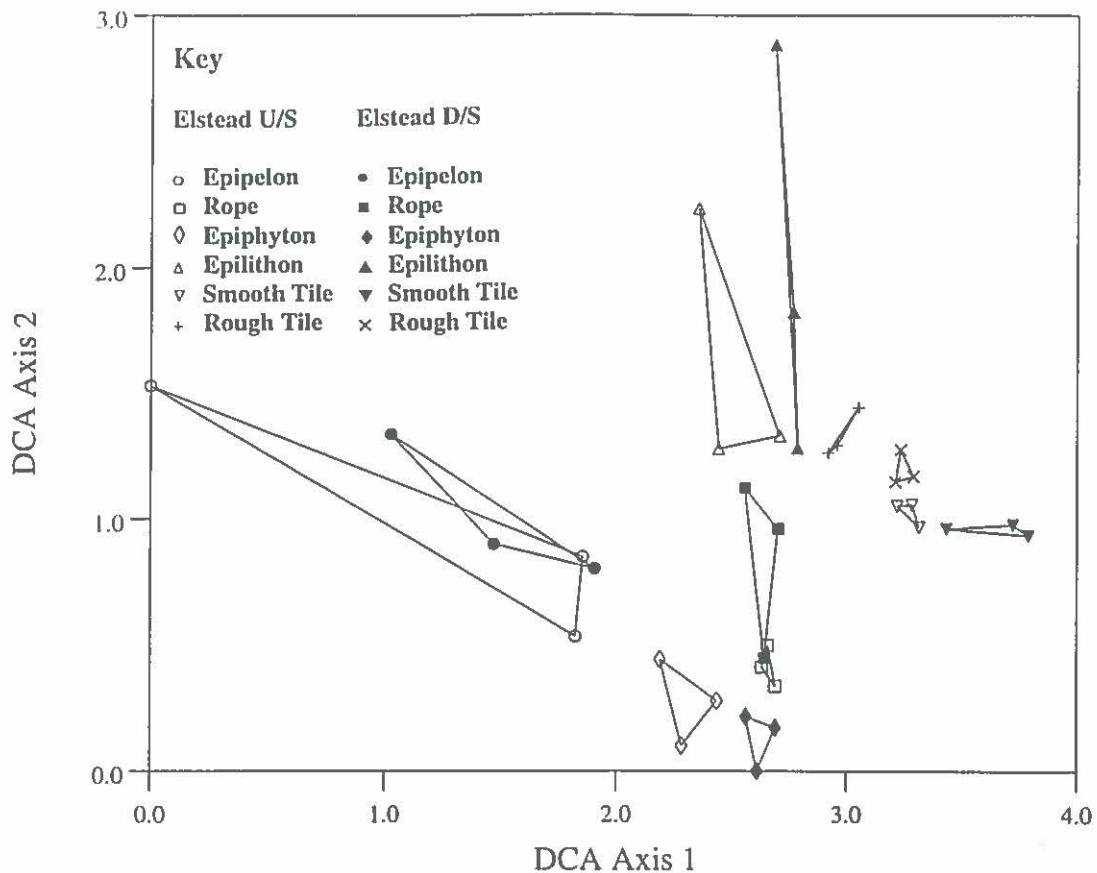


Figure 2. DCA ordination showing the different substrata at the two Elstead sites

The proximity of any two points on the DCA plot equates to their relative similarity in species assemblage. The total variation is therefore considered to be represented by the spread of points on the plot. The first axis is separating the epipelton from the artificial smooth tiles and axis 2 is separating the epilithon from the epiphyton samples. The variation within the epipelton and epilithon samples is high whereas the other samples all show a much closer relation. The proximity of the rough tile samples suggests it is closer to the natural epilithon community. The rope however plots centrally on the diagram and can therefore be considered to be more representative of all the habitats. Of the natural samples the epiphyton shows the least variation. This habitat is not easy to keep constant between the majority of sites and is therefore of limited use in monitoring studies. A further reason for it not being used is its low diversity (fig. 3)

As well as looking at sample variability the diversity of a community is also an important consideration. The more diverse a sample is then the more reliable a numerical assessment of trophic status, based on species indicator values (optima), will be. The Hill's N2 values (fig. 3) give a figure for the effective number of species occurring in a sample. Figure 3 shows that the epilithon supports the most diverse diatom flora within the natural substrata. The smooth tile, which shows very little within sample variation, has a low diversity. It is in fact characterized by a small number of generalist taxa with wide tolerances e.g. *Achnanthes minutissima*, *Gomphonema parvulum* and *Nitzschia paleacea*. The rough tile and rope both support a greater effective number of species than the natural epilithon.

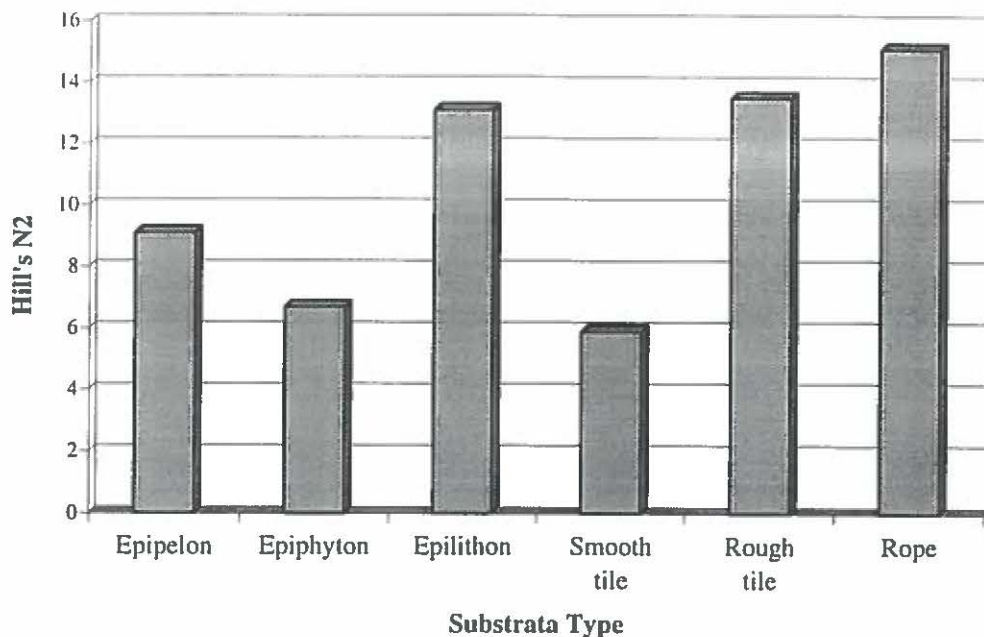


Figure 3. Mean Hill's N2 diversities for the different substrata

All these data are from counts made from cleaned diatom material. The material which was examined live showed a further advantage of using artificial substrata. Figure 4 shows the mean ratio of live to dead cells from rough tile and epilithon samples taken from the Elstead sites as well as two other sites on the River Wey under investigation at the same time. It is clear that the artificial tile samples support a much higher number of living diatoms than the natural epilithon. The assumption that this is true of all the artificial substrata is currently untested.

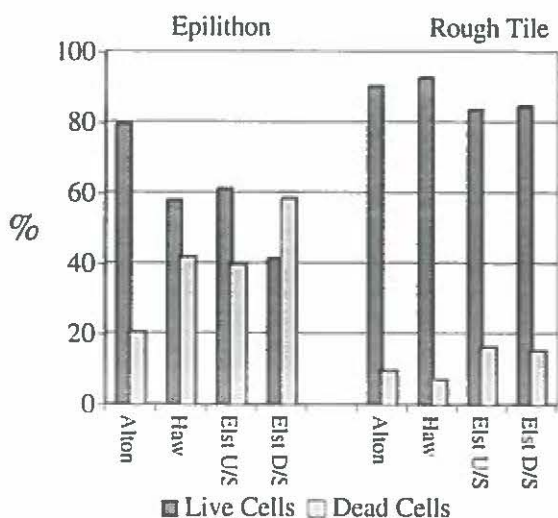


Figure 4. Live:dead cell ratios

A final consideration in the use of artificial substrata is their ease of use and success of recovery. In a recent survey of 58 river sites in, and around, the Thames catchment rough tiles and rope samples were left for one month. Deployment was the same as that described in table 1. Table 2 summarizes the recovery success of the two artificial substrata types following the one month period. Furthermore of these sites only 31 (53.4%) out of the 58 had what could be considered a good epilithic community. The rest had either no cobbles or those that were present were covered by a layer of silt.

Substrata	% Recovery
Rough tile	81
Rope	93

Table 2. Recovery of artificial substrata (n = 58)

### Discussion

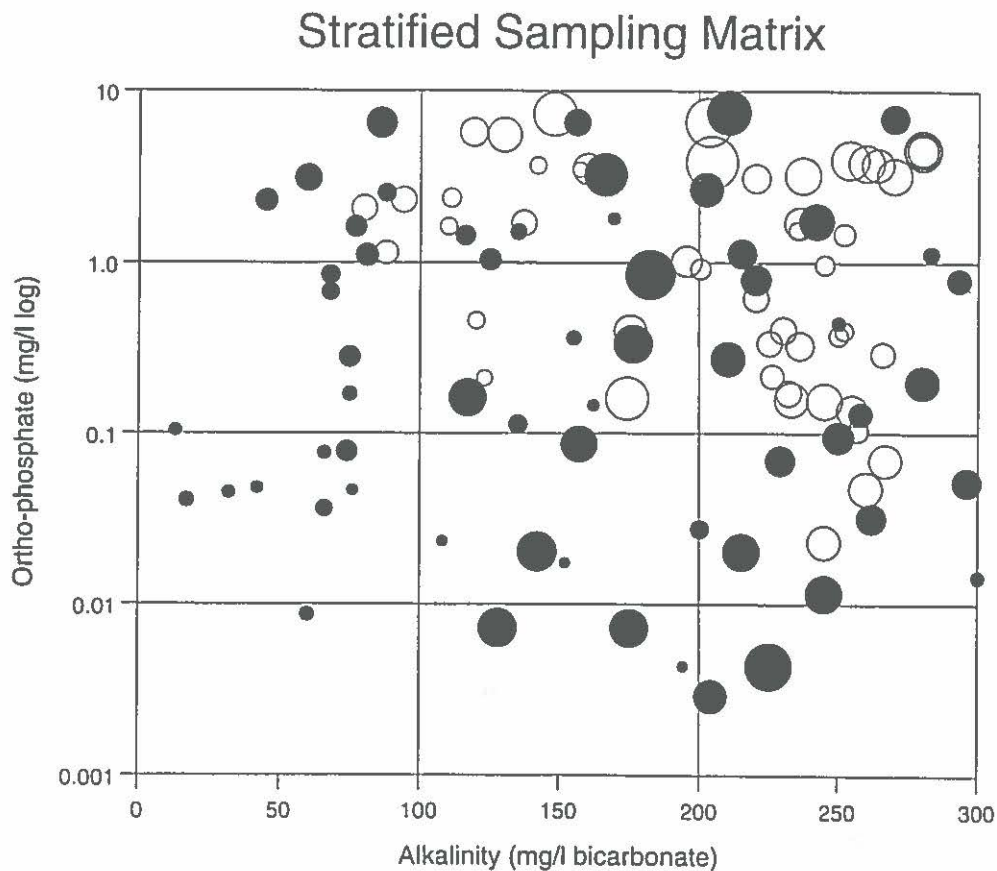
The results from this study demonstrate that the use of carefully selected artificial substrata can greatly decrease the sampling variability for river diatoms. The smooth tile is not considered a good artificial substrata due to it supporting a community of low diatom diversity, many of which are generalist taxa. Such species have little predictive value for assessing eutrophication

(Kelly & Whitton 1995). The rough tile and rope substrata, however, support a diatom flora that is both low in variability and high in diversity and are therefore considered here as reliable substrata for a diatom based monitoring system.

It was originally intended that the artificial substrata would be able to provide a reliable alternative to the natural epilithon where the latter was absent. There is however evidence that artificial substrata could further enhance diatom-based water quality assessments even where a natural epilithon does exist. The high number of dead cells in the natural community (fig. 4) should be considered a cause for major concern. A reliable monitoring system necessitates the diatoms to be living at the time of sampling. Dead diatoms may be a relic of a past pollution event or could be allochthonous in origin. The fine mucilage "forest structure" described for the epilithic diatom community (Round 1993) acts as a trap for inwashed, dead, diatom valves (Owen *et al.* 1979). This could be a particular problem when monitoring up stream and down stream of a

discharge if the diatoms from the clean site were to mask the living cells at the polluted site.

In lowland rivers, where the effects of eutrophication are most acute, there is an obvious need to maintain an effective monitoring strategy. Artificial substrata can enhance a diatom-based method of monitoring by providing a consistent substratum between very different river sites. The recent study of 58 sites allowed the successful deployment and retrieval of rope and rough tile substrata over a wide range of river habitats, from small fast flowing stony streams to the River Thames directly above Teddington Weir. With careful placement they can also be used in deep canals where the collection of any reliable natural substratum would not be possible. The high rate of recovery for artificial substrata, particularly of the rope samples, makes the logistics of their use very attractive when combined with the other advantages.



**Figure 5** Site selection matrix covering a wide FRP and total alkalinity gradient. The size of the circles denotes the concentration of Nitrate nitrogen ( $0.001 - 8.00 \text{ mg l}^{-1}$ ). The filled circles are the sites chosen for the study

## Development of the Artificial Substrata Methodology

Having developed an effective means of obtaining representative diatom samples from lowland rivers, work is currently being undertaken to assess the response of diatoms to trophic status on the two most reliable artificial substrata: rope and rough tiles. The 58 sites, mentioned above, cover a long gradient of phosphorus concentrations (and nitrate) over a range of differently buffered systems. The sites were chosen from an initial survey of 115 river sites in and around the Thames catchment. Water chemistry on these 115 sites was then fed into a Filterable Reactive Phosphorus (FRP) / alkalinity matrix (fig. 5). The final site selection was then made by taking, where possible, 6-7 sites from each section of the matrix.

The c. 60 sites are to be visited three times in total to gain two batches of diatom data from the rope and rough tile substrata. Water chemistry and physical data is also being collected. From this large data set it will be possible to obtain robust ecological information on the response of diatoms to the trophic gradient within rivers. This will form the initial data-phase for an ECRC based dynamic database (within Amphora (Beare 1996)) into which future artificial substrata diatom counts can be entered, along with chemistry data, to continually update the model.

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**Figure 5.** Rough tile and rope in place at Elst D/S



**Figure 6.** Rope in place at Elst D/S