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Bowes, M.J.; **Ings, N.L.**; **McCall, S.J.**; **Warwick, A.**; **Barrett, C.**; **Wickham, H.D.**; **Harman, S.A.**; **Armstrong, L.K.**; **Scarlett, P.M.**; **Roberts, C.**; **Lehmann, K.**; **Singer, A.C.** 2012 Nutrient and light limitation of periphyton in the River Thames: implications for catchment management. *Science of the Total Environment*, 434. 201-212. 10.1016/j.scitotenv.2011.09.082

Contact CEH NORA team at
noraceh@ceh.ac.uk

Nutrient and light limitation of periphyton in the River Thames: Implications for catchment management

Bowes, M.J[*a]., Ings, N.L[a,b]., McCall, S.J[a], Warwick, A[a]., Barrett, C[a]., Wickham H.D[a], Harman, S.A[a], Armstrong, L.K[a], Scarlett, P.M[a], Roberts, C[a]., Lehmann, K[a]. and Singer, A.C[a].

[a] *Centre for Ecology and Hydrology, Maclean Building, Crowmarsh Gifford, Wallingford, Oxfordshire, OX10 8BB, UK*

[b] *School of Biological and Chemical Sciences, Queen Mary, University of London, Mile End Road, London, E1 4NS, UK*

[*] *Corresponding author. E-mail address: mibo@ceh.ac.uk*

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Abstract

Soluble reactive phosphorus (SRP) concentrations in the River Thames, south east England, have significantly decreased from an annual maximum of 2100 µg l^{-1} in 1997 to 344 in 2010, primarily due to the introduction of phosphorus (P) removal at sewage treatment works within the catchment. However, despite this improvement in water quality, phytoplankton biomass in the River Thames has greatly increased in recent years, with peak chlorophyll concentrations increasing from 87 µg l^{-1} in the period 1997 to 2002, to 328 µg l^{-1} in 2009. A series of within-river flume mesocosm experiments were performed to determine the effect of changing nutrient concentrations and light levels on periphyton biomass accrual. Nutrient

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enrichment experiments showed that phosphorus, nitrogen and silicon were not limiting or co-limiting periphyton growth in the Thames at the time of the experiment (August – September 2010). Decreasing ambient SRP concentration from 225 µg l^{-1} to 173 µg l^{-1} had no effect on periphyton biomass accrual rate or diatom assemblage. Phosphorus limitation became apparent at 83 µg SRP l^{-1}, at which point a 25 % reduction in periphyton biomass was observed. Diatom assemblage significantly changed when the SRP concentration was reduced to 30 µg l^{-1}. Such stringent phosphorus targets are costly and difficult to achieve for the River Thames, due to the high population density and intensive agriculture within the Thames basin. Reducing light levels by shading reduced the periphyton accrual rate by 50 %. Providing shading along the River Thames by planting riparian tree cover could be an effective measure to reduce the risk of excessive algal growth. If the ecology of the Thames is to reach the WFD's "good ecological status", then both SRP concentration reductions (probably to below 100 µg l^{-1}) and increased shading will be required.

1 Introduction

The reduction of phosphorus (P) loading to UK rivers is seen as the key measure in reducing excessive algal growth and other problems associated with freshwater eutrophication, and thereby vital in delivering the "good ecological status" that is demanded by the European Union's Water Framework Directive (WFD). Many UK rivers have seen significant reductions in P concentration, due primarily to the introduction of phosphate removal at sewage treatment works (STW) (Bowes et al., 2009; Bowes et al., 2010b; Foy, 2007; Jarvie et al., 2002b; Neal et al., 2010a). These reduced P loadings have delivered the intended improvements in ecology in some rivers (Bowes et al., In press; Kelly et al., 2009), but many others have seen no change in either algal biomass or community structure (Kelly and Wilson, 2004; Neal et al., 2010b), because the current P concentrations are still in excess, and therefore do not limit algal growth rate.

If algal growth in a particular nutrient-enriched river is to be controlled by reducing phosphorus concentration, it is vital that catchment managers and policy makers know the threshold phosphorus concentration at which the algae become P-limited. If nutrient mitigation results in a river phosphorus concentration below this threshold value (termed the Phosphorus Limiting Concentration), algal growth will begin to decline, resulting in a potential shift in river ecology towards good ecological status (Hilton et al., 2006). If the phosphorus concentration remains above the P Limiting Concentration following mitigation, algal growth rate will continue unabated, and there is unlikely to be a change in river ecology. Previous studies have shown that Phosphorus Limiting Concentrations vary greatly from river to river, ranging from less than 20 μg l^{-1} SRP (Bothwell, 1985; Chambers et al., 2006; Popova et al., 2006; Welch et al., 1989) to ca. 100 μg l^{-1} or greater (Bowes et al., 2007; Matlock et al., 1999). Although the reduction of phosphorus loadings to rivers is seen as the main tool being used by government environmental and conservation agencies to control algal growth and improve ecological status in UK rivers, there are other parameters that can also affect algal accrual rate; in particular flow-velocity and light. Increasing river flow velocity can increase the scouring of epilithic and epiphytic biofilms from their substrates (Horner et al., 1990), thereby reducing shading of macrophyte leaves and 'cleaning' gravel substrates, thus increasing their utility as invertebrate habitat and potential fish spawning grounds. Increased flow velocity will also decrease residence times for phytoplankton (autotrophic organisms that are suspended within a water body), meaning that they have less time in the river to proliferate (Hilton et al., 2006). Light intensity within the river channel also affects the rate of algal growth (Hill and Fanta, 2008; Mosisch et al., 2001). Recent modelling studies on the River Swale, northern England, have suggested that reducing light levels in river headwaters by increasing riparian shading could be a more effective means of reducing phytoplankton growth than reducing phosphorus concentration (Hutchins et al., 2010).

In this paper, phosphorus and suspended chlorophyll-*a* concentration data for the River Thames from 1997 to 2010 are presented. These data were used to test the hypothesis that the improvements in water quality observed in other studies (Kinniburgh and Barnett, 2010; Neal et al., 2010a) have reduced phytoplankton concentration in the Thames. This paper then aimed to identify the Phosphorus Limiting Concentration of periphyton (attached biofilm) for the River Thames near Oxford, using within-river flume mesocosms. The flume mesocosms were also used to identify if the other major plant nutrients, nitrogen and silicon, were limiting or co-limiting biofilm development, and how increases in P, N and Si affect periphyton community structure. Finally, this paper aims to determine if decreased light intensity (equivalent to shading from riparian tree cover) will decrease periphyton accrual rates.

1.1 Catchment description

The River Thames is the largest river that is wholly in England, with a total length of 354 km and a catchment area (to the tidal limit at Teddington in south west London) of 9948 km^2 (Marsh and Hannaford, 2008). The river rises at Thames Head in Gloucestershire, and flows in an easterly direction into the North Sea, east of London (Figure 1). The Thames basin not only contains the UK's capital, London, but also many other major urban centres, including Swindon, Oxford, Slough, Maidenhead and Reading. The many STW associated with this high human population density (ca. 960 people km^{-2} (Merrett, 2007)) have a major impact on the water quality of the River Thames, with an estimated 50 % of the soluble reactive phosphorus (SRP) load derived from STW effluent between 1997 and 1999 for the relatively rural middle reaches of the Thames at Wallingford (Bowes et al., 2010b). Tertiary treatment has been installed at the 36 largest STW (serving approximately 2.7 million people) upstream of the tidal limit since 2003, resulting in an average 85 % reduction in phosphorus load from each sewage works (Kinniburgh and Barnett, 2010). This has resulted in significant reductions in SRP concentration in the River Thames and its tributaries during the 2000s (Kinniburgh and Barnett, 2010; Neal et al., 2010a). Over the coming decade, the population within

the Thames basin is likely to increase further, with the planned building of an extra 375 000 homes within the basin by 2016 (Environment Agency., 2009). This will greatly increase pressures on drinking water supplies, wastewater treatment, water quality and river ecology (Evans et al., 2003; Neal and Jarvie, 2005). These pressures are likely to be exacerbated by projected climate change scenarios that predict declining river flows and increasing water temperatures (Johnson et al., 2009).

Despite the high population density, much of the River Thames basin upstream of London is relatively rural (Environment Agency., 2009), with ca. 45 % of land area being classified as arable, 11 % woodland and 34 % grassland (Fuller et al., 2002). Only *ca*. 6 % of the catchment land cover was urban or semi-urban development. Agriculture is relatively intensive, and the resulting diffuse phosphorus, nitrogen and sediment losses will impact on water quality within the basin.

1.2 Study sites

The flume mesocosms experiments took place on the Seacourt Stream at Wytham, which is a small distributary / mill stream that is fed directly by the River Thames, just west of the city of Oxford (Figure 1). The Seacourt Stream was chosen to carry out nutrient limitation and shading experiments, rather than the River Thames itself, as the Thames is too deep for fieldworkers to safely operate the flumes. The Thames is also extensively used by leisure boats, and the large flume mesocosms used in this study would pose a hazard to this boat traffic. Twelve flumes (4 sets of 3 flumes) were installed *ca.* 50 − 80 m from the River Thames, along a relatively straight, uniform flowing section of river with a negligible amount of riparian shading. Maximum average river depth and width were *ca.* 1 m and 5 - 6 m respectively. Land use at the site was grassland, with sheep and cattle grazing. Simultaneous water sampling and analysis of the Seacourt Stream and Thames (just upstream of the confluence) showed that there was no observable change in nutrient concentrations taking place

within the Seacourt Stream itself, due to interactions with bed sediments or biota, and so the flume experiments were carried out on unaltered River Thames water.

The water quality and chlorophyll-*a* concentration of the River Thames at Wallingford (Figure 1) has been monitored at weekly interval from 1997 to 2002, 2006 to 2007 (Neal et al., 2010a), and from February 2009 until present, as part of the Centre for Ecology and Hydrology's Thames Initiative research platform. Water quality was also monitored at weekly intervals since February 2009 for the River Thames at Swinford (*ca.* 2 km upstream of the Seacourt Stream study site) (Figure 1).

2 Methodology

2.1 *Water quality analysis*

Samples of river water were taken manually at weekly interval from the main flow of the River Thames at Wallingford (1997 to 2010) and Swinford (2009 to 2010). Subsamples were filtered immediately in the field (0.45 um cellulose nitrate membrane filter, WCN grade; Whatman, Maidstone, UK), and analysed for nutrient concentration. Soluble reactive phosphorus was determined using the phosphomolybdenum blue colorimetry method of Murphy and Riley (1962), as modified by Neal et al., (2000). Samples were analysed within 24 hours, to minimise errors associated with sample instability (House and Warwick, 1998; Jarvie et al., 2002a). SRP is considered equivalent to bioavailable phosphorus (House, 2003). Dissolved reactive silicon concentration was determined by reaction with acid ammonium molybdate, to form yellow molybdosilicic acids. These were then reduced using an acidified tin (II) chloride solution to form intensely coloured silicomolybdenum blues, which were quantified spectrophotometrically using a Descrete Analyser (Auto Analyser 2; Seal Analytical, Fareham, UK) (Mullin and Riley, 1955). Nitrate-N concentration was analysed by ion chromatography (Dionex DX500). Chlorophyll-*a* concentration was determined by filtering a known volume of river water (*ca.* 500 ml) through a filter

paper (GF/C; Whatman, Maidstone, UK) and extracting the pigment using a solvent. The quantity of chlorophyll-*a* was then determined spectrophotometrically. The River Thames samples from 1997 to 2007 were extracted using ethanol (Neal et al., 2006), and from 2009 onwards, 90 % acetone was used for the pigment extraction (Marker et al., 1980). Parallel testing of river samples by the two solvent extraction techniques showed there was no observable difference between the two chlorophyll methods.

2.2 Flume mesocosm experiments

Within-stream through-flow flumes were used in this study to produce a range of nutrient concentrations, flows and light levels, simultaneously, at a single point in the river (Bowes et al., 2010a) (Figure 2), allowing the effect of these perturbations on biofilm accrual rate within each flume to be investigated. The flumes were fabricated from polyvinyl chloride (PVC) sheeting. Each flume was 5 m long and 0.3 m wide, with gates at the upstream end, to control the flow rate of river water entering them. The flumes were grouped in sets of three, and secured within aluminium frames to minimise flexing. Floats were attached along the sides of each set of three flumes, to maintain them at a constant water depth of 4 cm, irrespective of the changing water depth in the river. Because the flumes are floating at a constant depth within the water column, approximately 0.5 m above the bed sediment, they were not colonised by invertebrates, which would have a major effect on the amount of biofilm accrual that is observed during the experiments, due to grazing. The flumes were secured in place in the river by sliding them over metal poles that were driven into the river bed. Each flume had a 3 cm deep and 15 cm long sump, two thirds of the way down the flume (Figure 2), to collect chemical precipitate (from phosphorus reduction treatments used during the Experiment 1 (described below)) and river debris that entered the flume, preventing it from entering the lower section of the flume. Unglazed ceramic tiles (7 cm x 7 cm) were placed in this downstream section of each flume, to act as substrates for periphyton growth. Periphyton is a complex community of algae, cyanobacteria, heterotrophic microbes and associated detritus that produces a biofilm on within-river

surfaces. The rate of periphyton colonisation on new surfaces such as macrophyte leaves has been identified as the key process in ecological community degradation, associated with stream eutrophication (Hilton et al., 2006).

2.2.1 Effect of phosphorus concentration changes on periphyton (Experiment 1)

A phosphorus manipulation experiment was begun on the 18th August 2010. Six flumes were deployed in the Seacourt Stream at Wytham, in a river stretch with no shading from riparian vegetation and relatively uniform flow. The flumes were deployed in clusters of three, with a control and two nutrient treatments within each cluster. The flow-control gates at the upstream end of each flume (Figure 2) were adjusted until a water velocity of 0.10 m s^{-1} (measured using a Valeport model 801 electromagnetic flow meter (Valeport Ltd., Totnes, UK)) was produced in the middle section of each flume. One flume in each cluster received no chemical additions, and therefore had unmodified Thames river water running through it. These acted as experimental controls. Treatments were randomly assigned to the four remaining flumes, to simultaneously produce a range of phosphorus concentrations, spanning the ambient river concentration at the time of the experiment (Table 1). One of the flumes received a continuous addition of concentrated potassium dihydrogen orthophosphate solution into the upper section of the flume, to produce a *ca.* 60 % increase in the average ambient phosphorus concentration (which was 205 μg l^{-1} at the start of the experiment). The three remaining flumes had the SRP concentration in the incoming river water reduced, using the P-stripping methodology developed by Bowes and co-workers (2007). These flumes received continuous additions of a concentrated iron (II) sulphate solution. This added iron solution reacted with the soluble, bioavailable phosphate in the incoming river water, forming a non-bioavailable precipitate of $Fe_3(PO_4)_2$ (Reynolds and Davies, 2001; Suschika et al., 2001), thereby reducing the SRP concentration within the flume. The iron addition rates were adjusted until the SRP concentrations in the incoming river water (measured in the lower part of the flumes, where the tile substrates were to

be positioned) were reduced to approximately 75, 30 and 20 % of the current ambient SRP concentration. These phosphorus and iron additions were delivered to the upstream end of the flumes (to allow the chemical treatments to fully mix with the incoming river water and to allow time for the iron – phosphorus precipitation reaction to occur before the water reached the downstream periphyton-monitoring section of the flumes) from stock solutions on the river bank via pipes, using a multi-channel peristaltic pump.

When the required SRP concentrations were achieved, unglazed ceramic tiles were placed in the lower section of each flume, to provide substrates for periphyton growth. The SRP concentrations were monitored two to three times per day. Water samples (60 ml) were taken from the lower section of the flumes (where the ceramic tiles were positioned), immediately filtered through a 0.45 μm cellulose nitrate membrane filter (WCN grade; Whatman, Maidstone, UK) and analysed for SRP concentration (within 30 minutes of sampling) using a Micromac 1000 field autosampler / analyser (Systea Analytical Technologies, Anagni, Italy). Parallel investigations during this experiment and in previous flume studies (Bowes et al., 2010a; Bowes et al., 2007) confirmed that filtering the iron-dosed river water stopped the phosphorus stripping reaction, thereby allowing the true SRP concentration overlying the periphyton to be quantified. A randomly allocated tile was removed from each flume at two day intervals, to determine periphyton biomass by chlorophyll-*a* analysis.

After nine days (27th August, 2009), most of the tiles had developed a thick periphyton biofilm layer, and sloughing appeared to be imminent. The six remaining tiles in each flume and associated periphyton were carefully removed, and returned to the laboratory for chlorophyll-*a* analysis and algal community identification / quantification. These replicate tiles were used to provide information on within-flume variability in periphyton accrual rates and algal community structure.

2.2.2 Effect of increasing nutrient concentrations on periphyton (Experiment 2)

On the 18th August 2010, approximately 30 m upstream of the phosphorus manipulation experiment described above, a second flume experiment was undertaken, to investigate if periphyton in the River Thames was phosphorus, nitrogen or silicon limited / co-limited. As with Experiment 1, this study was conducted in two clusters of three flumes, one flume in each cluster randomly assigned to receive no chemical additions to the incoming river water, serving as an experimental control. The remaining four flumes had one or more of their P, N, and Si concentrations in the incoming river water increased, by the continuous addition of concentrated solutions of potassium dihydrogen orthophosphate (KH_2PO_4), sodium nitrate ($NaNO_3$) and sodium silicate solution ($Na_2O_7Si_3$) to the upstream end of the flume (Table 1). The treatments in these four flumes were: N, P, P + N, and P + N + Si. The nutrient enrichment treatments allocated to each flume are given in Table 1. The P and N stock solutions were made by dissolving KH_2PO_4 and $NaNO_3$ in deionised water. The silicon stock solution was produced by adding sodium silicate solution (dissolved in concentrated NaOH) to the alkaline river water from the study site, to minimise silicon precipitation within the stock solution. The openings in the gates at the upstream end of each flume were adjusted to give a standard flow velocity of 0.08 m s^{-1} in the middle of each flume. Unglazed ceramic tiles were then placed in the lower part of each flume to act as periphyton growth substrates. Water samples were taken from the lower section of each flume and immediately filtered through a 0.45 μm cellulose nitrate membrane filter (WCN grade; Whatman, Maidstone, UK). SRP analysis was carried out daily on site (at the University of Oxford's Wytham Field Station Laboratories) using a Micromac 1000 field nutrient analyser (Systea Analytical Technologies, Anagni, Italy). Silicon and nitrate samples were taken every two to three days, returned to the Centre for Ecology and Hydrology (CEH) Wallingford laboratories and analysed within 24 hours.

A randomly selected tile was removed at 2 day intervals throughout the experiment, for chlorophyll-*a* analysis. The experiment was terminated after 9 days, as some of the tiles were showing signs of having lost some periphyton biomass due to sloughing. The remaining tiles (six per flume) were removed and analysed for chlorophyll-a concentration and algal composition analysis.

2.2.3 Effect of light intensity on periphyton (Experiment 3)

Light intensities were measured in direct sunlight, and a range of dappled tree shade and full tree shade, using a 1 metre long light probe (SunScan, model SS1), around midday on a sunny day in September 2010. Dappled and full tree shade reduced the light intensity by 46 and 71 % respectively. Sheets of plastic mesh (greenhouse shading material) were then layered above the light meter, until shading levels equivalent to that of partial and full tree shading were obtained. These numbers of mesh layers were then placed over the downstream sections of the flumes, to recreate the light intensities equivalent to these different levels of tree shading. Previous studies have successfully used similar approaches to investigate light limitation of periphyton (Hill et al., 2009). Each set of three flumes contained an unshaded, intermediate shaded (equivalent to dappled tree shade) and fully shaded (equivalent to full tree canopy cover) flume. The positions of these shading treatments within each set of three flumes were allocated randomly (Table 1).

On 7th September, 2010, twelve flumes (four clusters of three flumes) were deployed at two points along a 30 m stretch of the Seacourt Stream, as described in Experiment 2. This third experiment aimed to examine the impact of light shading and flow velocity on periphyton biomass accrual and algal composition. Six of the flumes (two clusters of three) had the flow velocity of the incoming water set to 0.10 m s^{-1},(same as in Experiment 1) and the remaining six (30 m downstream of the first six flumes) had a higher velocity of 0.15 m s^{-1}, which was the highest consistent flow that could be achieved by opening up the flow control gates in each flume. Fourteen unglazed ceramic tiles were

placed in the lower section of each flume, underneath the shading material. Combined light / temperature loggers (HOBO pendent logger; Onset Computer Corporation, Massachusetts, USA) were placed alongside the tiles, to monitor levels of shading, and to determine if this shading reduced the water temperature within the flumes. The loggers were cleaned at two day intervals, to remove the colonising periphyton that was covering the light sensor, as this would cause errors in light intensity readings. Only light intensity readings taken after sensor cleaning were used to infer the effect of shading in each flume. Two tiles were removed from each flume at 2 day intervals, to determine chlorophyll-*a* concentration and algal community composition. The experiment was ended on the 17th September, 2010 (after ten days), as sloughing of periphyton from the tiles appeared to be imminent. The remaining six tiles in each flume were removed for subsequent chlorophyll-*a* and algal composition analysis.

2.3 Periphyton biomass analyses

The periphyton biomass that had accrued on each tile was estimated by chlorophyll-*a* analysis. The entire periphyton biofilm was washed and scraped from each tile, using 300 ml of deionised water. The resulting suspension was homogenised by vigorous stirring, and a 40 ml sub-sample was taken and filtered through a GF/C grade filter paper (Whatman Ltd., Maidstone, UK). The filtered sub-samples were then extracted overnight at 4°C in 90 % acetone and the quantity of chlorophyll-*a* in each sub-sample was quantified spectrophotometrically (Marker et al., 1980). The total quantity of chlorophyll-*a* on each tile was then back-calculated.

The total mass of periphyton and sediment covering the tile substrates was also quantified, by filtering another 40 ml sub-sample of the 300 ml homogenised suspensions (described above) through ashed, pre-weighed GF/C filter papers. The filter papers were then dried overnight at 60°C before being cooled to room temperature in a desiccator and reweighed. The dry weight within each sub-sample

was then used to calculate the dry mass on each tile (g cm^{-2}). Following this, filter papers were ashed at 500°C for two hours in a muffle furnace (model AAF 1100; Carbolite Ltd., Hope, UK). After cooling overnight at 60°C, filter papers were reweighed and the Ash Free Dry Mass (AFDM) on each tile back-calculated (which is equivalent to the organic content of the biofilm). Autotrophic Index was calculated by dividing the AFDM by the chlorophyll-a concentration (APHA., 1995). This gives an indication of the numbers of autotrophs in the biofilm, relative to heterotrophs and detritus.

For Experiment 3 (where replicate treatments were employed), a one-way analysis of variance (ANOVA) was used to test whether the different shading treatments in each flume had a significant effect on chlorophyll-*a* concentrations. *Post-hoc* Tukey's multi comparison test followed significant ANOVA ($p < 0.05$), to differentiate between mean chlorophyll-*a* concentrations. If the chlorophyll concentration data were not normally distributed or the variances were unequal (tested using Levene's test), the data were log-transformed prior to analysis. Differences in periphyton biomass under different flow conditions in Experiment 3 were analysed using a two sample *t*-test.

2.4 Diatom community analysis

Diatom communities were investigated on three tiles per flume, sampled at the end of each experiment. The biofilm was scrubbed off each individual tile using a toothbrush and rinsed into a clean tray using deionised water. Samples were transferred to 50 ml plastic tubes, kept cold and dark in the field and then frozen immediately on return to the laboratory. Defrosted samples were thoroughly mixed, before *ca.* 10ml of sample was placed in a beaker and cleaned using the 'hot peroxide method' (Kelly et al., 2001). Hydrogen peroxide solution (30 %) was added to each sample and these were then gently heated on a hotplate until the samples had turned clear. A few drops of 10 % hydrochloric acid were added to remove carbonates, before samples were rinsed three times in deionised water. Samples were mounted onto slides using Naphrax (refractive index of 1.73, Brunel Microscopes Ltd, Chippenham, UK).

Frustules were identified according to the methods described in Kelly et al., (2001), using the keys of Krammer & Lange-Bertalot (2004a, b, 2007a, b) and Kelly et al. (2005). On each slide counting ceased once 300 frustules belonging to non-planktonic taxa had been identified. For Experiment 1, diatoms from all flumes were identified. In Experiment 2, diatoms from the N addition, N,P,Si addition and one control flume were identified (flumes 1 to 3; Table 1) and in Experiment 3, diatoms from flumes 4 to 12 were identified, to give three replicates of each level of shading (Table 1).

To investigate the effect of nutrient concentration on diatom community composition, the trophic diatom index (TDI) was calculated for each sample in Experiments 1 and 2 using the nutrient sensitivity and indicator values and the formula given in Kelly et al., (2001). Possible outcomes can range from 0 to 100, with a 100 indicating extremely high nutrient concentrations. In addition, the percentage of the diatom community composed of motile species was calculated.

To characterise any differences in community composition between treatments, multivariate analyses were performed using CANOCO version 4.5 (Ter Braak, 1988-2003). Exploratory detrended correspondence analyses (DCA) indicated that gradient lengths were short (less than 2) and this, together with an inspection of species responses, suggested that linear methods would be most appropriate (Leps and Smilauer, 2002). Redundancy analysis (RDA) was used to examine relationships between species and environmental data (SRP concentrations and levels of shading, input as nominal variables for Experiments 1 and 3 respectively and for Experiment 2 concentrations of SRP, N-nitrate and dissolved reactive silicon input as continuous variables) and Monte Carlo permutation tests were used to test the significance of the environmental variables. Principal components analysis (PCA) was used to show the variation in diatom community structure across the treatments. Changes in species diversity were also quantified using the Shannon diversity index.

3 Results and discussion

3.1 Changes in River Thames water quality

Phosphorus concentrations have decreased significantly in the River Thames at Wallingford since the late 1990s (Figure 3). The maximum SRP concentrations observed in 1997 and 1998 were 2100 and 1788 µg l^{-1} respectively, with mean annual concentrations of 1320 and 789 µg l^{-1}. The annual maximum and mean SRP concentrations declined to *ca.* 800 and 420 µg l^{-1} in each of the following 3 years (1999 to 2001). Maximum and mean total phosphorus (TP) concentrations also declined from 2792 and 1461 µg l^{-1} in 1997 to 844 and 471 µg l^{-1} in 2001, respectively. Similar sudden declines in SRP concentration in 1999 have also been observed in Environment Agency (EA) water quality data for a monitoring site 2 km downstream of Oxford at Radley, and was attributed to the introduction of phosphorus removal at Swindon STW (with a population estimate of 199 000) in the upper Thames catchment (Kinniburgh and Barnett, 2010).   Further reductions in SRP concentration have been observed from 2002 to 2010 (Figure 3), with maximum and mean concentrations in 2010 of 344 and 202 µg l^{-1} respectively, and due principally to the introduction of P removal at 36 of the largest STWs in the catchment (Kinniburgh and Barnett, 2010). There has also been a 26 % reduction in annual average NO_3-N concentration during this monitoring period, decreasing from 8.9 mg l^{-1} in 1998 to 6.6 mg l^{-1} in 2010.

However, this major improvement in water quality and reduction in trophic status observed in this study has not delivered the expected reduction in phytoplankton concentration. In fact, there has been an almost four-fold increase in chlorophyll-*a* concentrations in recent years, with a maximum observed concentration of 87 µg l^{-1} between 1997 and 2002, increasing to 328 µg l^{-1} in 2009 (Figure 2). This observation is at odds with the conclusions of Kinniburgh and Barnett (2010), who's chlorophyll concentration data from the years 1994 and 2006 implied that phytoplankton

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concentrations had begun to decline in the Thames, despite the orthophosphate concentrations still far exceeding the values they thought to be limiting to algal growth. The chlorophyll concentration in 2006 was actually the lowest observed within this study, with a maximum of less than 12 µg l^{-1}, despite the P concentration being similar to that in 2009. By combining the observations from these CEH and Environment Agency (EA) studies, and in particular the nutrient and chlorophyll-*a* data from 2009-2010, it is now clear that declining SRP concentrations have not been reduced to algae-limiting levels, and other factors that control phytoplankton concentration (such as river ecology and food-web interactions, light, water temperature and flow) must be the cause of the low algal biomass observed in 2006 and the major algal bloom in 2009, rather than nutrient availability.

3.2 Effect of phosphorus concentration changes on periphyton

The SRP concentrations observed in each flume throughout the P manipulation experiment (Experiment 1) are shown in Figure 4. The SRP concentration of the two control channels (equivalent to the ambient concentration in the Seacourt Stream) throughout the nine days of the experiment varied between 203 and 260 µg l^{-1}, with an average concentration of 225 µg l^{-1}. The flume receiving soluble phosphate additions varied from 297 to 480 µg l^{-1}, with an average SRP concentration of 373 µg l^{-1}, which was equivalent to a 66 % increase in ambient SRP concentration over the course of the experiment. The three flumes receiving the iron sulphate additions had the SRP concentration of the incoming river water reduced by 24, 63 and 87 %, giving average SRP concentrations throughout the experiment of 171, 83 and 30 µg l^{-1} respectively. The nitrate-N concentration during this experiment was 5.2 mg l^{-1}, giving N:P ratios ranging from 384:1 to 31:1 for the 30 and 373 µg l^{-1} respectively. The N:P ratio in the River Thames at the study site was 51 at the time of the experiment.

The rates of periphyton accrual in each flume (estimated by chlorophyll-*a* concentration) through the course of the experiment are shown in Figure 5, and the final average concentrations of chlorophyll-*a*

produced at the range of average SRP concentrations at the end of the experiment (day 9) are shown in Figure 6. Figure 5 shows that the amount of chlorophyll-*a* on the tiles in one of the control flumes had started to decrease between day 8 and 9, corroborating the field observations during the experiment that periphyton biomass in some flumes had reached its maximum by day 9, and that biofilm sloughing was beginning to take place. After nine days, the quantities of chlorophyll-*a* produced by the P-addition flume, one of the control flumes, and the iron-addition flume with its average SRP concentration reduced to 171 µg l^{-1}were all similar, with chlorophyll-*a* concentrations ranging from 12.6 to 13.2 µg cm^{-2} (Figure 6). The dry mass of periphyton and sediment that had accumulated in these flumes varied between 21 and 23 mg cm^{-2}, with an organic content (quantified by AFDM) of 15 to 16 %. The other control flume (with an SRP average of 225 µg l^{-1}) had a lower quantity of periphyton biomass (chlorophyll-*a* concentration of 11 µg cm^{-2}), due to the sloughing identified in Figure 5. The two flumes that had their SRP concentrations reduced to 83 and 30 µg l^{-1} had average chlorophyll-*a* concentrations of 9.8 and 9.4 µg cm^{-2} respectively, equivalent to a 25 to 30% reduction when compared to the *ca.* 13 µg cm^{-2} of the other treatments, and a reduced dry mass of *ca.* 18 mg cm^{-2}. Autotrophic Index (AI) values varied from 190 to 253, which indicate that the biofilm community is relatively well balanced between heterotrophic and autotrophic conditions (Ameziane et al., 2002). The highest AI value was from the flume with an average SRP concentration of 30 µg l^{-1}., indicating that the algal population has been suppressed at this low phosphorus concentration.

These results show that phosphorus is not limiting the rate of periphyton accrual in the River Thames at the time of this study. A 60 % increase in ambient SRP concentration from *ca.* 225 to 373 µg l^{-1} did not increase the rate of periphyton accrual, showing that phosphorus is in excess for periphyton growth. This explains why there has not been a reduction in phytoplankton biomass in the River Thames, despite a reduction in average annual SRP concentration from *ca.* 2000 µg l^{-1} in the late 1990s to 202 µg l^{-1} in 2010. Reducing the SRP concentration to 171 µg l^{-1} also had no effect on the

biomass of periphyton produced. Phosphorus limitation was only observed in flumes with SRP concentrations of 83 and 30 µg l⁻¹, but this only reduced the periphyton biomass by *ca.* 25 %. Therefore, the phosphorus limiting concentration in this part of the River Thames is between 83 and 171 µg l⁻¹. Similar periphyton limitation studies on the River Frome, Dorset and the River Kennet (a major tributary of the River Thames) identified phosphorus limiting concentrations of *ca.* 90 and 60 µg l⁻¹ SRP respectively (Bowes et al., 2010a; Bowes et al., 2007). As a reduction in SRP concentration to 83 µg l⁻¹ only resulted in a minor reduction in the periphyton biomass and accrual rate, it is likely that River Thames SRP concentration will probably need to be reduced to below 100 µg l⁻¹ during the ecologically-active spring and summer period before a reduction in algal biomass and a potential improvement in ecological status would be observed.

In contrast to the chlorophyll-*a* results, diatom community composition only significantly altered from that present at ambient SRP concentrations once SRP concentrations were reduced to 30 µg l⁻¹, suggesting that the Phosphorus Limiting Concentration was between 30 and 83 µg l⁻¹. This is illustrated in Figure 7, with the three replicate samples from the 30 µg SRP l⁻¹ flume markedly separated (largely along axis 1) from the other samples. Further support is provided by the RDA in which this treatment was the only one which significantly explained any variation (variation explained 32.4 %, $F = 7.68$, $p = 0.002$) in the diatom communities. This sudden shift in community composition at an SRP concentration of 30 µg l⁻¹ is also apparent in terms of both diversity and the percentage of motile cells. This flume contained a greater diversity of diatoms and a reduced proportion of motile cells compared to all other flumes in this experiment (Table 3). The diatom community was characterised by an increase in the abundance of taxa in class 3 of the TDI, described as 'species favoured by intermediate concentrations of nutrients' (Kelly et al., 2001) and this was balanced by a slight reduction in taxa in sensitivity class 4 described as 'species favoured by high concentrations of nutrients'. Taxa in sensitivity class 3 that were more common under the lowest P concentrations included *Suriella brebissonii, Navicula capitoradiata* and *Cocconeis placentula,*, whereas this flume

contained a lower relative abundance of *Nitzschia acicularis* and *N. capitellata*, which were taxa from sensitivity class 4 (Figure 7).

The TDI index values did not show any marked difference among treatments, which may suggest that this tool is relatively insensitive at SRP concentrations greater than *ca.* 50 µg l^{-1}. However, it should be noted that the communities examined here were relatively young (9 days old), and TDI is usually applied to more mature and established communities. Despite this, the communities contained high algal standing crops and were sampled at a time at which sloughing appeared imminent, suggesting that the biofilm had reached its maximum biomass. The apparent lack of sensitivity of the TDI results to a magnitude change in SRP concentration may suggest that factors other than SRP concentration may control the diatom community composition at this site. These factors could include the deposition of algal cells from upstream, high propagule pressure, and the large amount of suspended sediment present within the river water (*ca.* 10 mg l^{-1}). High rates of deposition of algal cells seems unlikely to account for the differences in chlorophyll concentrations and diatom community composition observed here as planktonic diatom species (mainly *Cyclotella* spp*., Stephanodiscus* spp. and *Aulacoseira* spp.), only accounted for 6.0 ± 0.4 % of all frustules identified across this study. These centric diatoms attained high abundances within the phytoplankton in the Thames at Wallingford at the time of this experiment. Greater differences in chlorophyll concentrations within the shading experiment (see below) also indicates that algal growth was taking place within the flumes and thus communities were not just a reflection of high rates of colonisation within a large river.

Thus, chlorophyll-*a* concentrations responded more readily to the reduction in P concentrations than diatom community structure. Minor changes in algal community structure in response to a reduction in water P concentration has also been reported by Steinman et al. (1991), who attributed it to the relatively modest change in P concentration used in their study (from *ca.*7 to 3 µg l^{-1}). However, in

this study (as in a number of other studies e.g. Hill et al., 2009; Rosemond et al., 2000) a large range in P concentration was used, and it suggests that the diatom community is resistant to major changes in water phosphorus concentrations, at least over the short time scales investigated. Furthermore, it is likely that the species pool within the River Thames is dominated by taxa adapted to high nutrient concentrations.

3.3 Effect of increasing nutrient concentrations on periphyton

The nutrient concentrations observed in each flume during Experiment 2 are given in Table 2. Flumes receiving phosphorus additions had the SRP concentrations in the incoming river water increased from 235 to *ca.* 300 µg l^{-1}; equivalent to an increase of between 25 and 30 %. Nitrogen addition flumes had their average NO_3-N incoming river water increased by 15 %, from 5.2 to *ca.* 6.0 mg l^{-1}. The flume receiving additions of all three nutrients (Flume 2) also had its dissolved reactive silicon concentration increased by 15 %, from the average ambient river concentration of 3.05 mg l^{-1} to 3.5 mg l^{-1}. A greater increase in ambient dissolved silicon concentration in this flume was not possible, due to rapid precipitation when the silicon stock solution entered the flume.

The quantities of chlorophyll-*a* that had accrued on the tile substrates in each flume are shown in Figure 8. There was no apparent difference between any of the nutrient-addition treatments and the controls. Furthermore, the highest average quantities of chlorophyll-*a* were actually observed in the control flumes receiving unmodified river water. This shows that periphyton at the study site are not limited, or co-limited by any of the three major plant nutrients; P, N or Si, during the experiment. From analysing the water quality monitoring data obtained from the River Thames at Swinford and at Wallingford, nitrate concentrations through 2010 were at their minimum concentration during late August (when the experiment took place). Therefore, periphyton at the study site will not be nitrogen-limited or co-limited throughout the entire year. In contrast, phosphorus and silicon

concentrations in the Thames at Swinford and Wallingford were approaching their annual maximums in August 2010, and were both at a minimum in April and May, and so there is a possibility that these nutrients may become limiting or co-limiting at other times of the year at the study site, and in particular during the spring period when concentrations are lowest and algal biomass (as measured by suspended chlorophyll-*a*) is usually at its highest.

In agreement with the chlorophyll data, there was also no impact of increasing nutrient concentrations on the composition of the diatom communities; in the RDA, concentrations of P, N and Si were not significant (all canonical axes: $F = 0.94$, $p = 0.54$). Furthermore, TDI values were similar across treatments (river water = 77.07 ± 0.10; N addition = 77.81 ± 0.94; P, N and Si addition = 78.44 ± 0.97) as were the percentage of diatoms that were motile (river water = 72.67 ± 0.51; N addition = 73.78 ± 1.68; P, N and Si addition = 73.33 ± 3.08).

3.4 Effect of light intensity on periphyton

The effect that the shading materials had on light intensities reaching the ceramic tile substrates are shown in Figure 9. The tiles in the unshaded flumes (in direct sunlight for most of the day) received maximum light intensities of between 26 000 to 58 000 Lx through the course of the experiment, with an average daily maximum of 44 000 Lx. The average light intensity during daylight hours was 15 700 Lx. The flumes that had intermediate shading had maximum light intensities ranging from 16 000 to 40 000, and an average daytime light intensity of *ca.* 10 000. This intermediate, partial shading reduced the maximum daytime light intensity in these flumes to *ca.* 27 000 and 10 000, equivalent to reducing direct sunlight by *ca.* 35 to 40 %. The fully shaded flumes had maximum light intensities of between 7 000 and 19 000 Lx (Figure9), and an average daytime light intensity of 4500 Lx, which was equivalent to reducing direct sunlight by *ca.* 70 %. These reductions in direct sunlight monitored within the flumes were closely comparable with those observed under partial and full tree canopy

cover of 46 and 71 % respectively, prior to the start of the experiment. Flume daily water temperatures (measured at hourly interval) ranged from 14 to 17 ^{o}C throughout the study. The shading treatments had little effect on water temperature within the flumes, with all flumes having an average temperature of 15.8 ^{o}C.   However, the unshaded flumes had their daytime maximum temperatures increased by *ca.* 0.4 ^{o}C.

The flumes in direct sunlight accrued significantly more periphyton than the shaded flumes (F = 12.16, p = 0.003) (Figure10). Intermediate shading (equivalent to dappled tree shading) reduced the chlorophyll-*a* concentration by 30 % in water velocities of 0.10 m s^{-1}, and by 20 % at 0.15 m s^{-1}. The fully shaded flumes (equivalent to full riparian tree canopy cover) reduced the accrued periphyton biomass by 34 to 56 %, compared to flumes in full sunlight. The Autotrophic Index values for the unshaded flumes in the low and high flow velocity flumes (235 and190 respectively) were much lower than the two fully shaded flumes (380 and 358), suggesting that reduced light levels were limiting the algal population. For all three shading treatments, there were no significant differences between high and low flow-velocities (unshaded; t = 0.03, p = 0.978), (partial shade; t = -1.51, p = 0.182), (full shade; t = 0.86, p = 0.416), showing that this small difference in water velocity of 0.05 m s^{-1} had little effect on periphyton accrual rates. Further studies are needed to investigate the impact of flow velocity on biofilm accrual rate, using more representative ranges of velocities that are experienced by epiphytic and epilithic biofilms within river channels.

Light intensity also influenced diatom community composition (Figure11); the community growing in the full shade flumes were significantly different (RDA, F = 8.52, p = 0.002, 25 % of variance in species data explained) from that found at higher light intensities. However, there was no difference in the mean TDI scores for the three treatments (no shade: 76.30 %, intermediate shade: 77.73 %, full shade: 77.38 %). Shaded flumes contained more diverse communities with a lower proportion of

motile diatoms (Table 4). Shading was also found to reduce the proportion of motile diatoms in a recirculating flume study by Lange et al., (2011). As in the current study (Figure11), the motile diatom *Nitzschia palea* was less abundant in the shaded treatments, whereas the adnate diatoms *Cocconeis placentula* and *Achnanthidium minutissimum* occurred at higher abundances under reduced light (Lange et al., 2011). Motile diatoms are generally at an advantage within thick biofilms (e.g. biofilms growing in full sunlight), where their motility allows them to access light and nutrients more effectively than attached diatoms.

3.5 Implications for future management of the River Thames catchment

The water quality of the River Thames has greatly improved since the late 1990s, and this seems to be directly attributable to the STW improvements that have been introduced within the catchment. Phosphorus concentrations in the River Thames at Wallingford have been reduced ten-fold between 1997 and 2010, and nitrate concentrations have also been reduced by 26 % during the same period. However, water quality monitoring within this study, in conjunction with earlier data sets (Neal et al., 2010a), have shown that phytoplankton biomass has greatly increased in the late 2000s, with the summer of 2009 having a maximum chlorophyll-*a* concentration of 328 μg l^{-1}, a factor of ten higher than those seen in 1997 to 2002. Other studies have regularly observed similar maximum chlorophyll-*a* concentrations (of between 250 to 350 μg l^{-1}) in this part of the River Thames in the period 1980 to 1994 (Kinniburgh et al., 1997), prior to STW improvements. This is of great concern, as the reduction of phosphorus concentration is seen by UK policy makers and catchment managers as one of the main tools in delivering the good ecological status required under the WFD, and algal biomass is seen as the key symptom of eutrophication.

This study has shown that despite the dramatic reductions in nutrient enrichment, phosphorus and nitrate concentrations in the River Thames are still too high to limit or co-limit both phytoplankton and periphyton biomass. The use of the phosphorus stripping methodology within this study has shown that reducing the summer SRP concentration by a further 50 µg l^{-1} (to 171 µg l^{-1}) would still have no effect on algal growth rates and algal community composition. It is also likely that such a P reduction would have no observable effect on the river's macrophyte and invertebrate assemblages, which also need to be improved to comply with the WFD. Some reduction in periphyton accrual rate would be observed at an SRP concentration of 83 µg l^{-1}. These observations fit with the suggested average annual SRP targets to achieve good ecological status in the UK of 120 µg l^{-1} (UKTAG., 2008).   Average summer SRP concentrations approaching 30 µg l^{-1} would need to be attained before a change in algal community structure would be observed, but even this 87 % further reduction in SRP concentration would perhaps only reduce periphyton accrual rate by 25 %.

The River Thames catchment (like most catchments in lowland southern Britain) has high human population densities and intensive agricultural land use, and it is highly likely that attaining these stringent phosphorus concentration targets will bedifficult and costly. However, nutrient reduction is not the only tool available to catchment managers to improve ecological status. This study has shown that providing riparian shading in the upper Thames perhaps offers an alternative tool to control nuisance algal growth and improve ecological status. Periphyton growth rate could be reduced by *ca.* 50 % if the river channel was shaded from direct sunlight by riparian tree-cover. Similar observations have been made from studies of forested and open streams in Queensland, Australia (Mosisch et al., 2001) and USA (Greenwood and Rosemond, 2005; Jacobson et al., 2008). Modelling studies of the River Swale and River Ure in northern England have predicted 40 to 50 % reductions in phytoplankton biomass could be achieved by providing riparian shading (Hutchins et al., 2010). However, the response of algal communities to light and nutrients is often inter-dependent (Hill et al.,

2009; Rosemond et al., 2000), and manipulating both factors simultaneously within the River Thames could potentially lead to still further decreases in algal biomass, and this requires further investigation.

The large increases in chlorophyll-*a* concentrations observed in the River Thames in 2009 and 2010 are clearly not related to increases in nutrient loading, as these particular years had the lowest phosphorus concentrations observed over the 13 year water-quality monitoring period. The flume experiments within this study have also shown that nutrients do not currently limit or co-limit periphyton growth, and therefore, other factors that can control algal biomass (such as river ecology / food-web interactions, light, water temperature and flow velocity) must be the cause of both the low algal biomass observed in 2006 and the major algal blooms in 2009 and 2010, rather than nutrient availability. Determining the causes of these huge annual fluctuations in algal biomass are key to understanding the process of eutrophication, and how we can effectively manage our rivers in the future.

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Figure 7. The PCA biplot of diatom communities across the SRP concentrations in Experiment 1 (black circles = 30 µg l^{-1}, grey circles = 83 µg l^{-1}, black diamonds = 171 µg l^{-1}, white diamonds and white circles = 225 µg l^{-1} and grey diamonds = 373 µg l^{-1}). The primary axis explains 64.9 % of variation with the secondary axis explaining a further 18.7 %. Arrows indicate the direction of increase in abundance for the taxa with most influence (Coc pla = *Cocconeis placentula*, Gom par = *Gomphonema parvulum*, Nav capr = *Navicula capitatoradiata*, Nav cryc = *Navicula cryptocephala*, Nav str = *Navicula stroemii,* , Nav trip = *Navicula tripunctata*, Nit cap = *Nitzschia capitellata*, Nit pal = *Nitzschia palea*, Nit palc = *Nitzschia paleacea*, Rho abb = *Rhoicosphenia abbreviata*, Sur bre = *Surirella brebissonii*, Sur min = *Surirella minuta*, Syn uln = *Synedra ulna*, Try lev = *Tryblionella levidensis* and Try hun = *Tryblionella hungarica*).

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Figure 11. The PCA biplot of diatom communities across the three shading levels in experiment 3 (Black circles = full shading; Grey circles = partial shading; White circles = no shading). Scaling is focussed on inter-sample distances, and samples are labelled by flume number. The primary axis explains 33.4 % of variation with the secondary axis explaining a further 26.6 %. Arrows indicate the direction of increase in abundance for the taxa with most influence (Ach min = *Achnanthidium minutissimum*, Amp ova = *Amphora ovalis*, Amp ped = *Amphora pediculus*, Coc ped = *Cocconeis pediculus*, Coc pla = *Cocconeis placentula*, Fal mit = *Fallacia mitis*, Gom ang = *Gomphonema angustatum*, Nav cryn = *Navicula cryptotenella*, Nav lan = *Navicula lanceolata*, Nav trip = *Navicula tripunctata*, Nit aci = *Nitzschia acicularis*, Nit amp = *Nitzschia amphibian*, Nit cap = *Nitzschia capitellata*, Nit pal = *Nitzschia palea*, Nit sig = *Nitzschia sigmoidea*, Sur bre = *Surirella brebissonii*, Sur min = *Surirella minuta* and Syn par = *Synedra parasitica).*

Table 1. Nutrient and light treatments applied during flume experiments.

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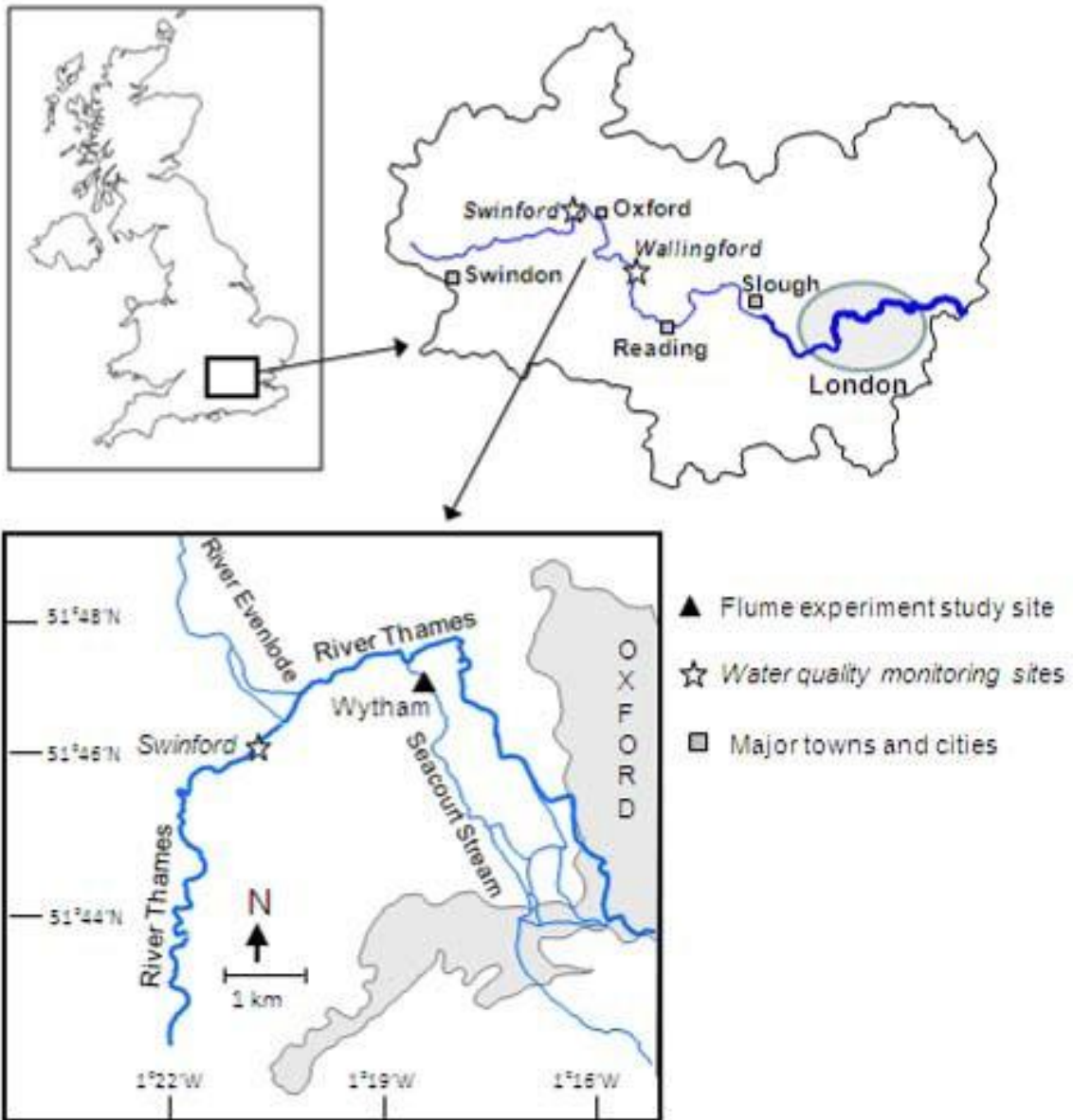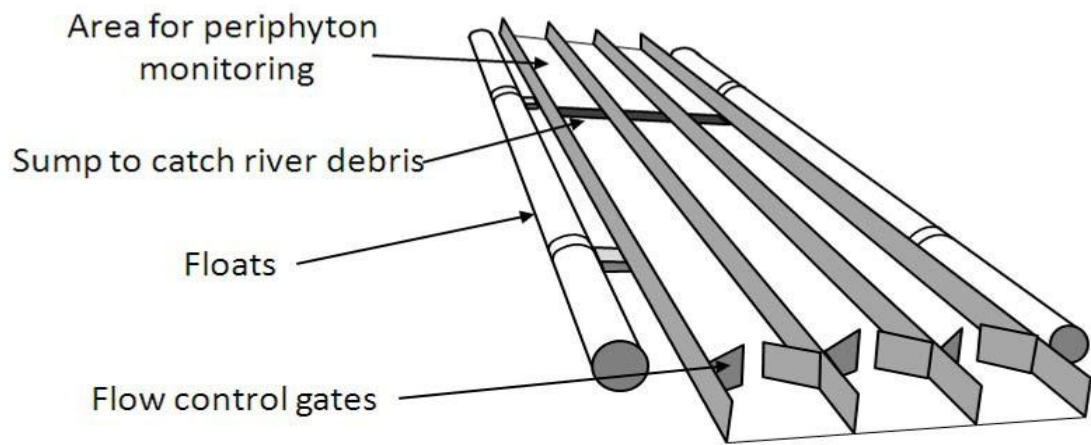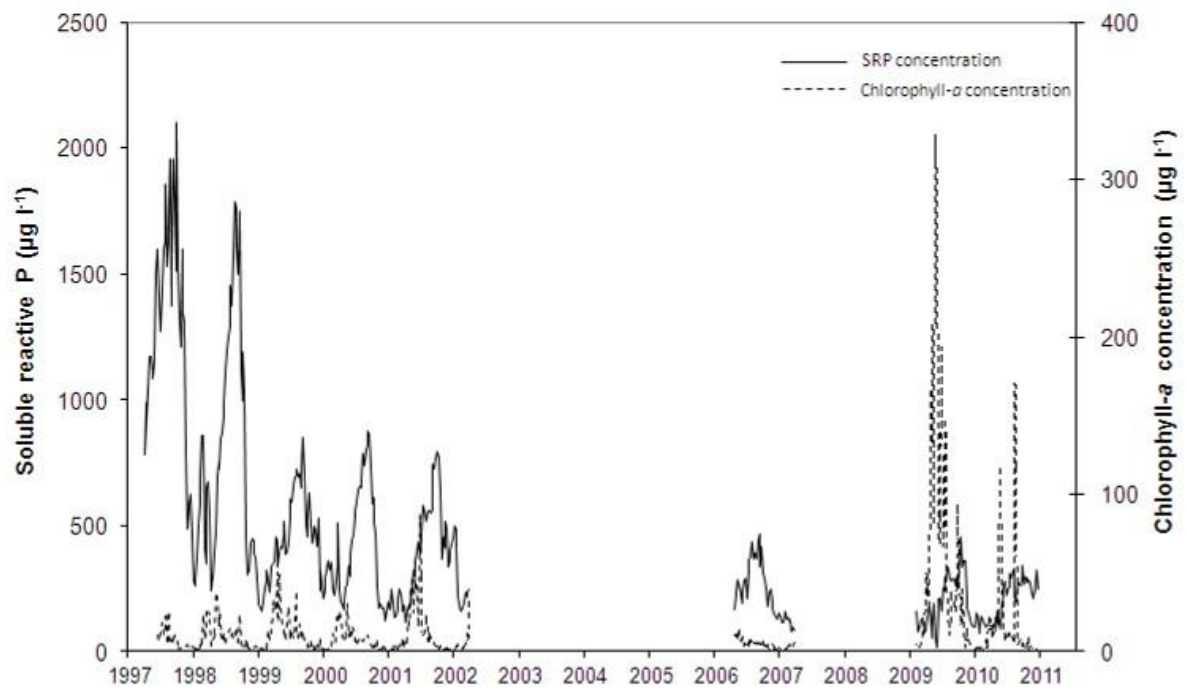
Figure 1

Area for periphyton monitoring

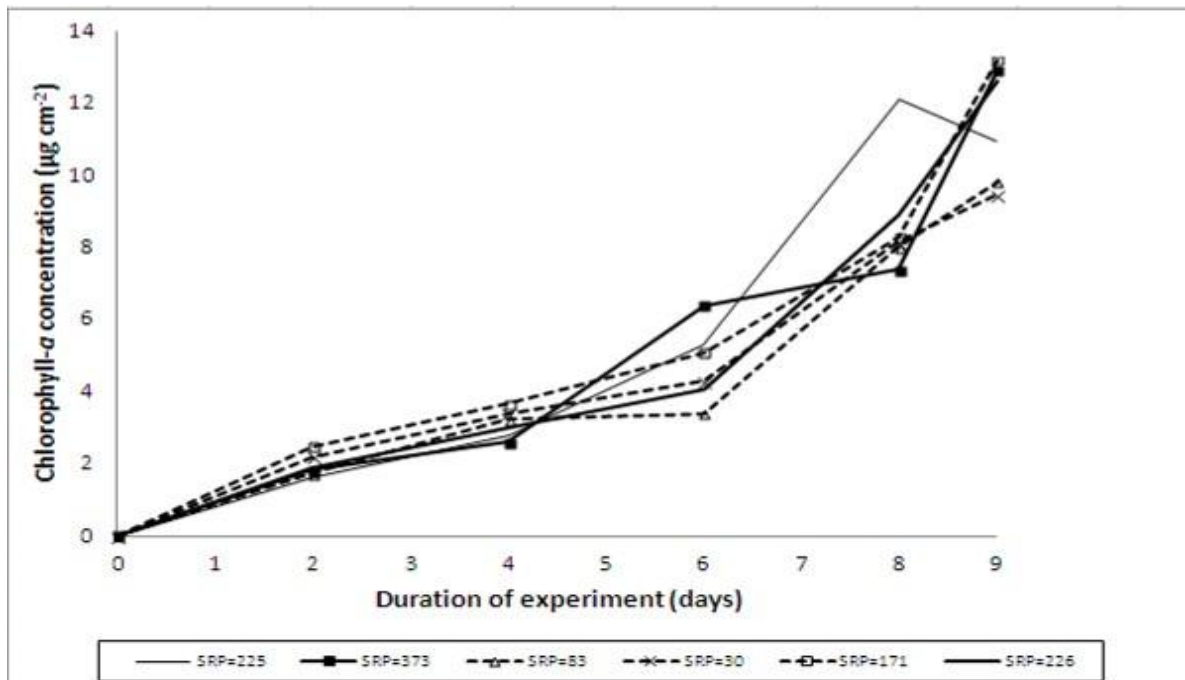Sump to catch river debris
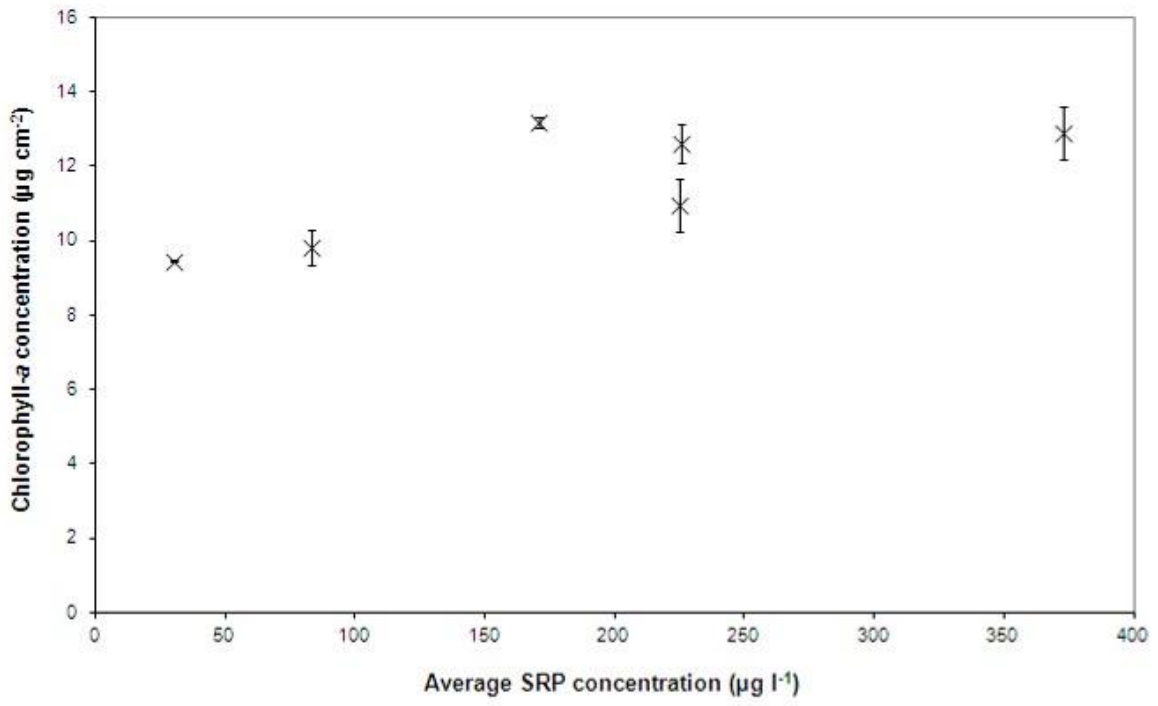
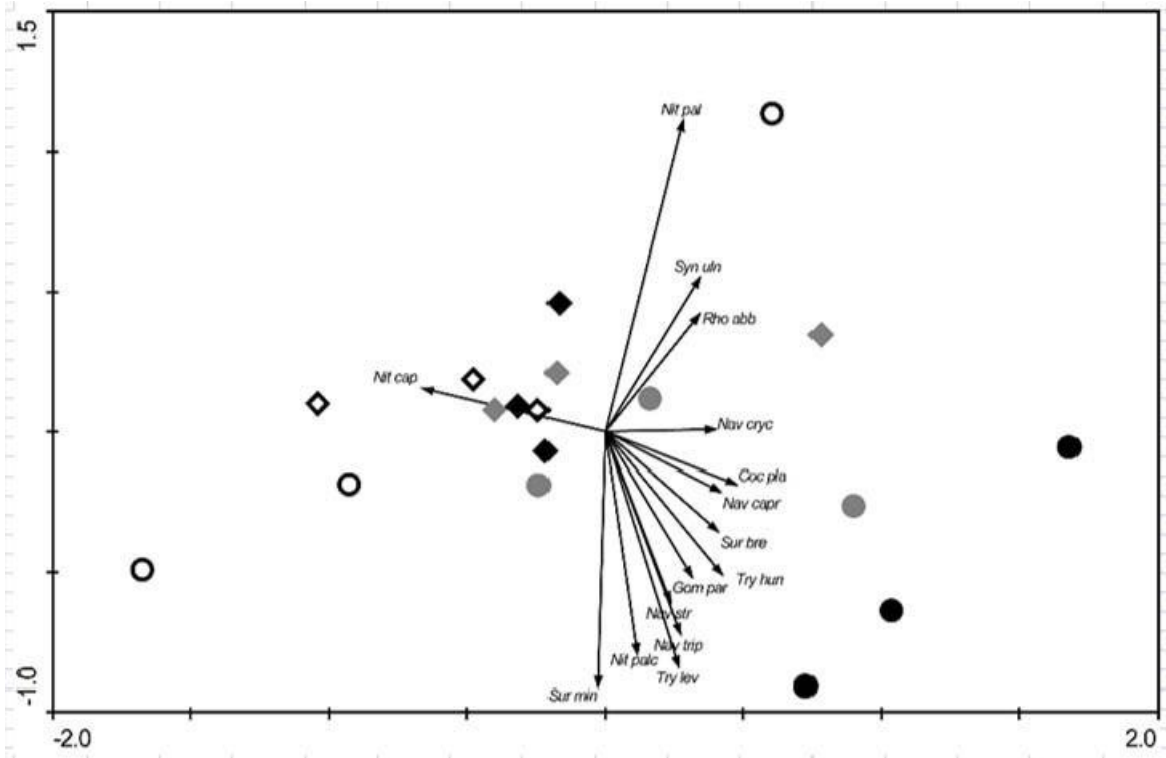Floats

Flow control gates
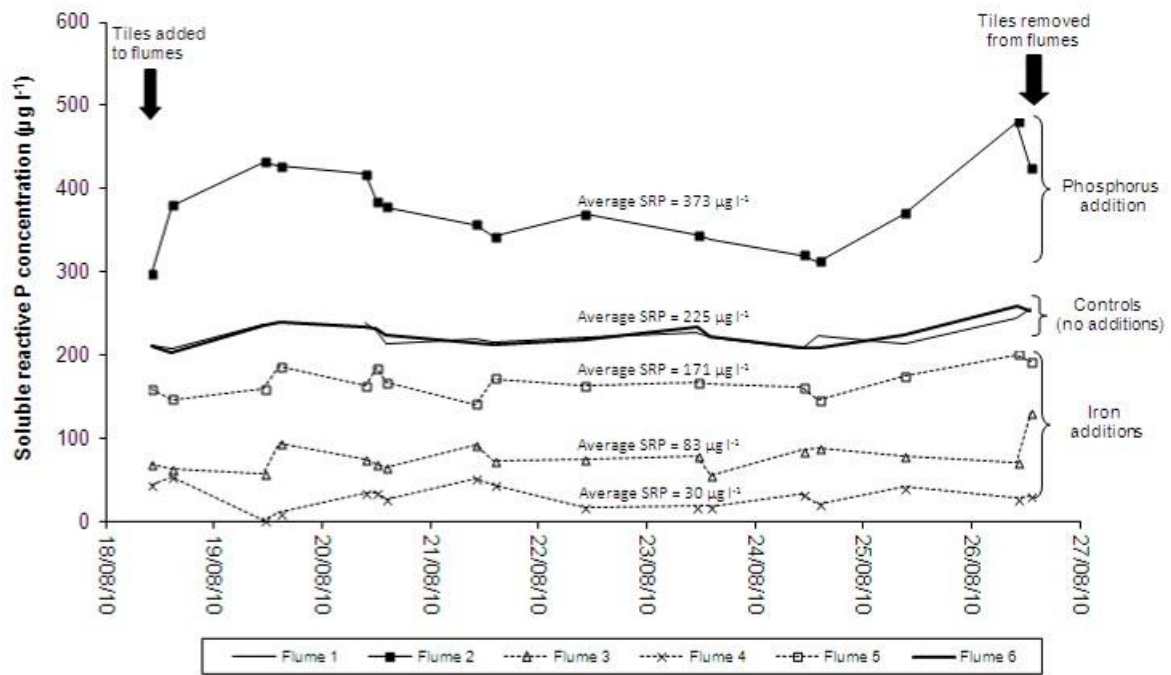
Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

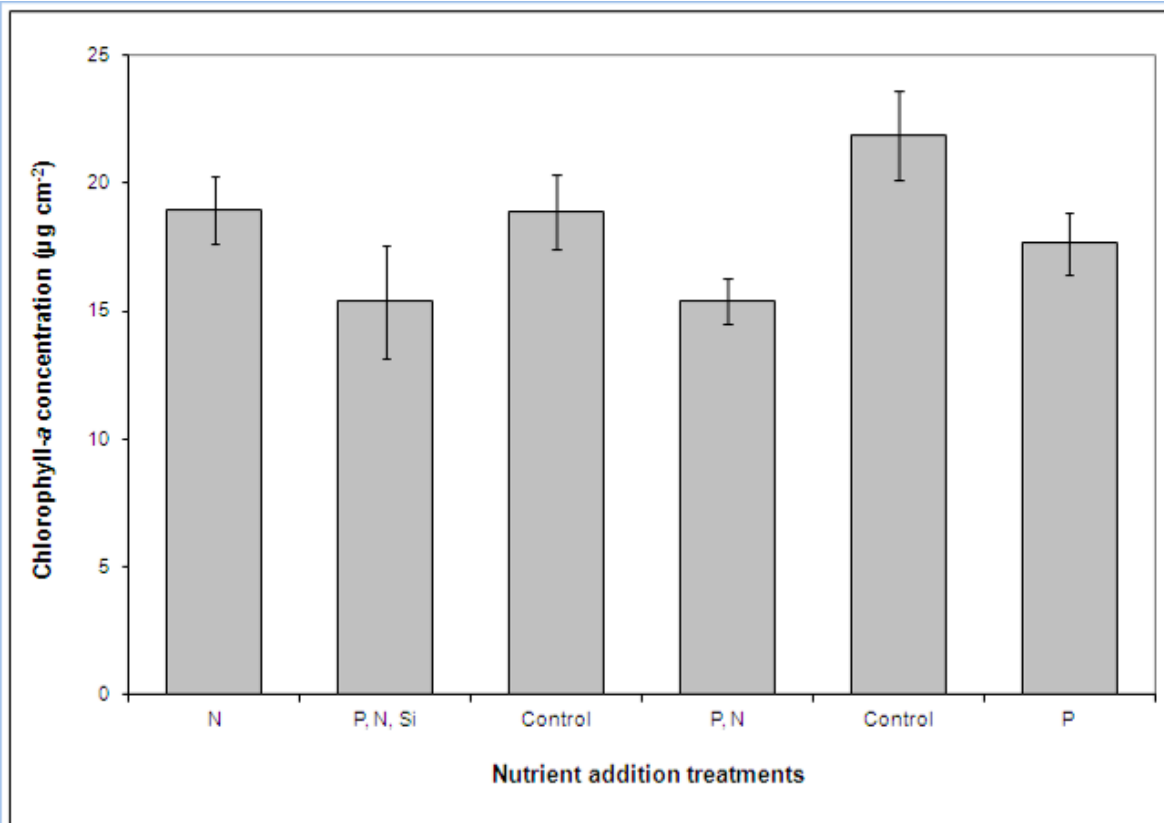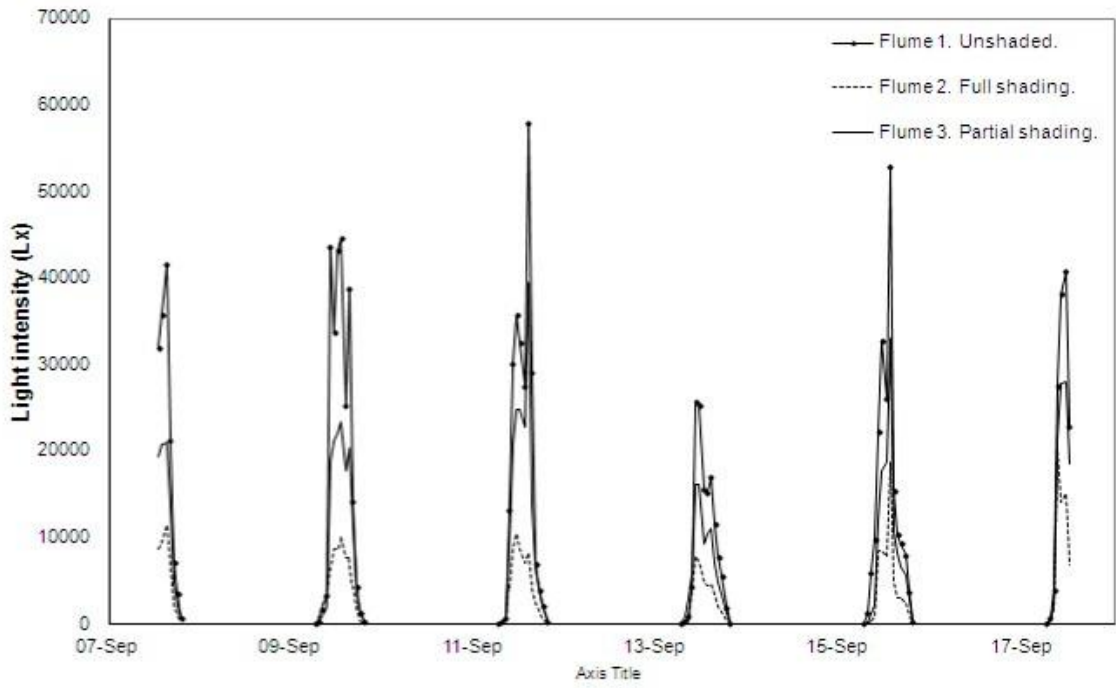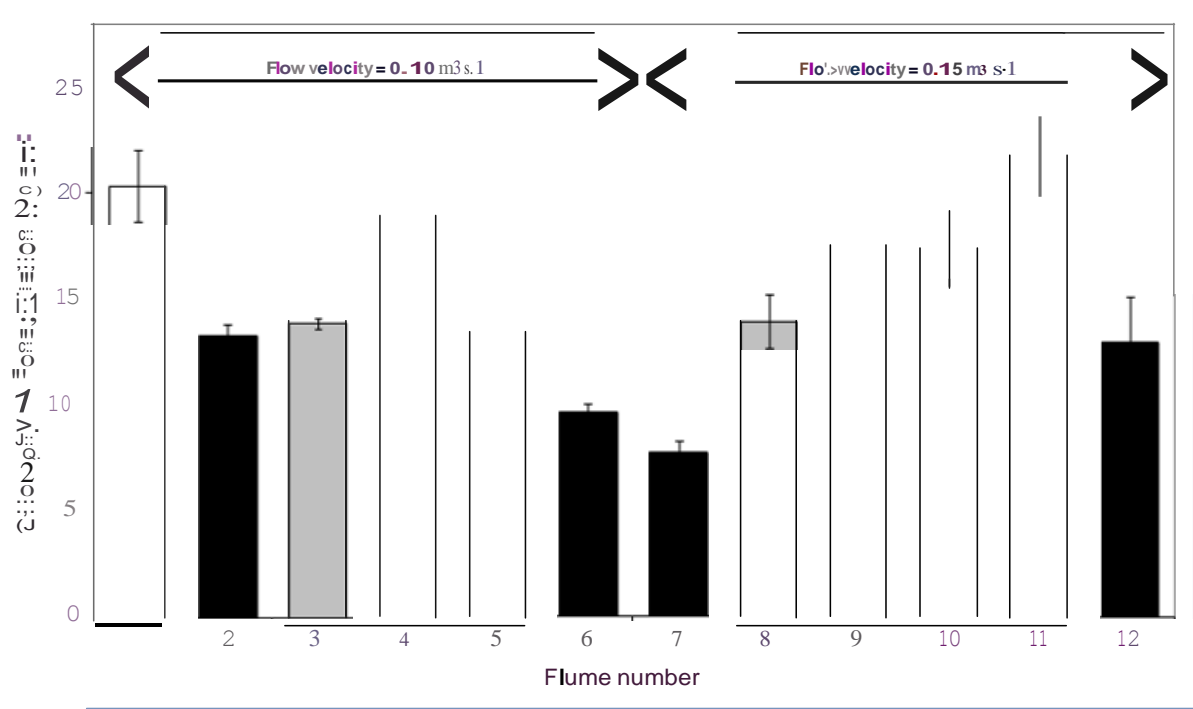**Figure 7**

Figure 8

Figure 9

Figure 10
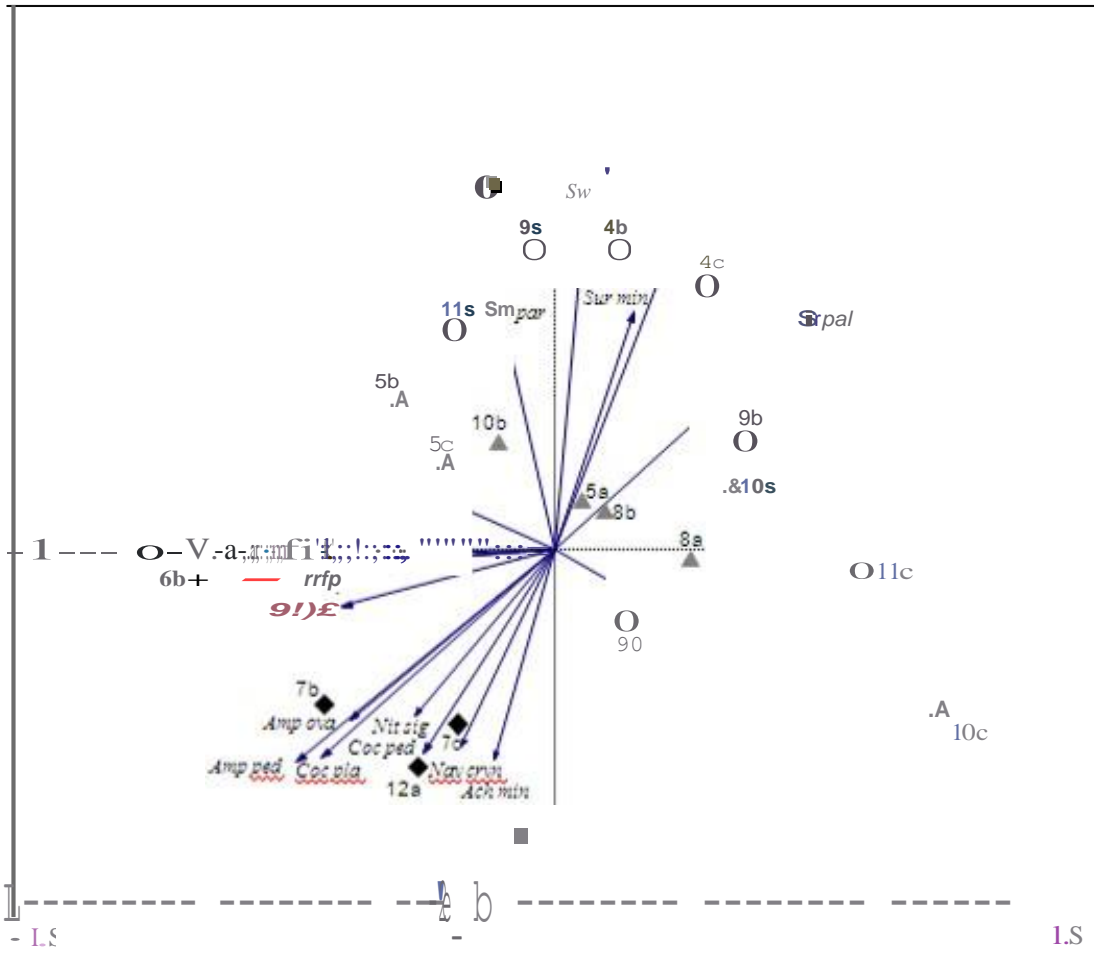
Figure 11

Experiment number	Dates (Duration)	Flume number	Treatment	Flow velocity (m s⁻¹)
Experiment 1	18th to 27th August 2010 (9 days)	1	Control	0.10
		2	P addition (80x increase in initial P concentration)	0.10
		3	Iron addition (30X decrease in initial P concentration)	0.10
		4	Iron addition (70X decrease in initial P concentration)	0.10
		5	Iron addition (20X decrease in P concentration)	0.10
		6	Control	0.10
Experiment 2	18th to 27th August 2010 (9 days)	1	N addition	0.08
		2	P, N and Si addition	0.08
		3	Control	0.08
		4	P and N addition	0.08
		5	Control	0.08
		6	P addition	0.08
Experiment 3	7th to 17th September 2010 (10 days)	1	No shading	0.10
		2	Full shading	0.10
		3	Intermediate shading	0.10
		4	No shading	0.10
		5	Intermediate shading	0.10
		6	Full shading	0.10
		7	Full shading	0.15
		8	Intermediate shading	0.15
		9	No shading	0.15
		10	Intermediate shading	0.15
		11	No shading	0.15
		12	Full shading	0.15

Table **1**

Flume number	Treatment	Soluble reactive phosphorus	Nitrate-N	Dissolved reactive silicon
		9 r'	mg l"¹	mg l"¹
1	N	238	6.05	3.05
2	P, N, Si	303	5.85	3.50
3	Control	235	5.19	3.06
4	P, N	287	5.81	3.06
5	Control	233	5.18	3.05
6	P	302	5.18	3.05

Table 2

Soluble reactive phosphorus (I)	Flume	Trophic diatom index	Motile diatoms (o/o of frustules)	Shannon diversity index
30	4	76.15 ± 0.88	71.46 ± 0 24	3 03 ± 004
83	3	78.04 ± 0.69	82.02 ± 2.52	2.65 ± 0 11
171	5	81.59 ± 0.63	80.04 ± 2.22	2.49 ± 0.04
225"	1	77.45 ± 1.77	85.72 ± 1.15	2.27 ± 0.07
225•	6	78.14 ± 0.85	80.49 ± 1.76	2.39 ± 0.08
373	2	79.67 + 0.41	83.00 + 2.03	2.45 + 0.09

• river water concentration

Table 3

Treatment	Shannon diversity index	Percentage of diatoms that are motile
Unshaded	2.85 ± 0.02	83.71 ± 0.54
Intermediate shading	2.97 ± 0.06	81.48 ± 1.70
Full shading	3.24 ± 0.10	69.48 ± 1.82

Table 4