Periphyton biomass response to changing phosphorus concentrations in a nutrient-impacted river: a new methodology for phosphorus target setting

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Abstract: Nutrient modification experiments were conducted in streamside flumes to determine the concentration at which P limits algal growth in the mesotrophic River Frome, Dorset, UK. The soluble reactive P (SRP) concentration in each flume was either increased (by P addition), decreased (by precipitating P with iron(II) sulphate solution), or left unaltered (control), producing SRP concentrations ranging from 32 to 420 $\mu g \cdot L^{-1}$. Increasing the ambient SRP concentration did not increase epilithic algal growth, showing that the River Frome was not P limited at 109 μg SRP$\cdot L^{-1}$. In the P-stripped flumes, algal biomass declined as the SRP concentration fell below ~90 $\mu g \cdot L^{-1}$, with a 60% biomass reduction at <40 μg SRP$\cdot L^{-1}$. Phosphorus-diffusing periphytometers deployed in the P-stripped flumes confirmed that reduced rates of algal growth were due to P limitation rather than a physical effect of $FeSO_4$ addition. The ~90 $\mu g \cdot L^{-1}$ maximum P-limiting concentration is likely to be similar for comparable nutrient-impacted rivers. This iron-stripping approach expands the existing river nutrient-enrichment methodology so that it can be used in nutrient-impacted rivers and should allow catchment managers to produce knowledge-based P reduction targets prior to introducing remediation.

Résumé : Nous avons mené des expériences de modification des nutriments dans des canalisations en bordure du cours d'eau afin de déterminer la concentration à laquelle le P limite la croissance des algues dans une rivière mésotrophe, la Frome, Dorset, R.-U. Nous avons augmenté (par addition de P), diminué (par précipitation du P avec une solution de sulfate de fer(II)) ou maintenue inchangée (témoin) la concentration de P réactif soluble (SRP) dans chaque canalisation, ce qui a produit des concentrations variant de 32 à 420 $\mu g \cdot L^{-1}$. L'accroissement de la concentration de SRP ambiant ne fait pas augmenter la croissance des algues épilithiques, ce qui montre que la Frome n'est pas limitée par P à 109 μg SRP$\cdot L^{-1}$. Dans les canalisations à P réduit, la biomasse des algues décline lorsque les concentrations de SRP baissent sous ~90 $\mu g \cdot L^{-1}$; la réduction de biomasse est de 60 % lorsque les concentrations de SRP sont <40 μg SRP$\cdot L^{-1}$. L'utilisation de périphytomètres diffuseurs de P dans les canalisations à P réduit confirme que la diminution des taux de la croissance des algues est due à une pénurie de P plutôt qu'à un effet physique de l'addition de $FeSO_4$. La concentration limite maximale de P de ~90 $\mu g \cdot L^{-1}$ est vraisemblablement similaire dans des rivières comparables affectées par les nutriments. Notre méthode de réduction du P à l'aide du fer vient s'ajouter aux méthodologies existantes pour l'étude de l'enrichissement des rivières en nutriments; elle peut être utilisée dans les rivières affectées par les nutriments et elle devrait permettre aux gestionnaires des bassins versants de fixer des objectifs de réduction de P qui soient basés sur des études avant de mettre en place des mesures de mitigation.

[Traduit par la Rédaction]

Introduction

Much effort is currently being focused on reducing P and N loadings in nutrient-impacted freshwaters to improve environmental status and reduce the risk of eutrophication. Initiatives such as the Urban Wastewater Treatment Directive (European Economic Community 1991) were designed to produce significant reductions in P concentrations in the mainly urbanized lowland catchments across the European Union. However, the resulting step-changes in P loading following P removal from sewage treatment works often have no effect on algal community structure (Kelly and Wilson 2004), suggesting that P concentrations are not limiting (or colimiting) algal growth in many of these rivers. Catchment

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managers and policy makers need to focus their resources on implementing nutrient load reductions to rivers that would produce an observable environmental improvement and a reduction in nuisance algal biomass. However, the information that they require to make such decisions (such as what is the river's limiting nutrient and what target nutrient concentration should be set) is extremely limited.

One approach commonly used to determine which nutrient is potentially limiting algal growth is molar ratios of N to P in river water (Redfield 1958). However, many recent studies have questioned the effectiveness of this approach (Allen and Hershey 1996; Francoeur et al. 1999; Stelzer and Lamberti 2001). At the elevated nutrient concentrations present in many urbanized lowland rivers across Europe, algal biomass is likely to be light or temperature limited (Hilton et al. 2006), and so nutrient ratios may be of little value.

Nutrient-diffusing substrata have been used to determine which nutrient, if any, is limiting periphyton growth (Chessman et al. 1992; Francoeur et al. 1999; Matlock et al. 1999). These studies are purely quantitative, as the amount of nutrient enrichment cannot be quantified (owing to varying nutrient diffusion rates from the substrate (Scrimgeour and Chambers 1997) combined with changing river flow velocities). They are therefore unable to show how a specific change in nutrient concentration will affect the quantity of periphyton in a river. Other researchers have studied the effects of direct nutrient enrichment of streams (Elwood et al. 1981; Peterson et al. 1993; Sabater et al. 2005) and within-stream/streamside flumes (Bothwell 1985; Lohman et al. 1991; Stelzer and Lamberti 2001). Most of these studies have been based on predominantly rural catchments with low initial nutrient concentrations. This approach allows algal biomass response to be determined following specific increases in nutrient concentration. However, such experiments can only simulate the effect of nutrient concentration increases and not decreases.

To determine the actual concentration at which P no longer limits algal growth (the maximum P-limiting concentration), the initial river P concentration already needs to be below this concentration (i.e., P must be limiting for P enrichment to be an effective approach). As most lowland rivers in urbanized catchments already have high P loadings (Bowes et al. 2005*a*), nutrient-enrichment approaches often cannot be used to determine this P-limiting concentration. Rier et al. (2006) have addressed this weakness by decreasing stream nutrient concentrations using algal uptake in partially recirculating flumes, but this nutrient reduction approach is unlikely to be suitable for use in highly nutrient-impacted systems.

This study aims to develop a P-stripping technique to expand the existing methods of studying nutrient limitation in rivers, which can be used to simultaneously assess the effects of increasing and decreasing soluble reactive P (SRP) concentration on algal biomass. The ability to decrease SRP concentrations means that P limitation can, for the first time, be studied in highly nutrient-impacted rivers, where ambient P concentrations are in excess of algal growth limiting concentrations. This approach will allow the effects of specific P mitigation strategies to be quantified in terms of a direct impact on algal biomass. This study applies this new methodology to determine the SRP concentration at which P begins to limit epilithic algal growth for a mesotrophic river, the River Frome, Dorset, UK.

Materials and methods

Study area

The River Frome drains a predominantly chalk catchment of 414 km^2 area (Casey and Newton 1973) (Fig. 1). The land use within the catchment is primarily agricultural, mainly grassland and cereals (Casey et al. 1993). The town of Dorchester is the only significant urban area within the study reach. Ten sewage treatment works discharge treated effluent into the River Frome and its tributaries (Fig. 1). The largest of these serve the towns of Dorchester (population equivalent = 27 600), Wool (population equivalent = 8000), and Warmwell (population equivalent = 3900). The mean annual discharge of the river at East Stoke was 5.13 $m^3 \cdot s^{-1}$ in 1999 (Bowes et al. 2005*b*).

This research was conducted using experimental flumes located at the Freshwater Biological Association's Dorset River Centre in East Stoke, Dorset, UK (formally the Institute of Freshwater Ecology's River Laboratory). The research facility is located alongside the Mill Stream, which is a branch of the lower River Frome.

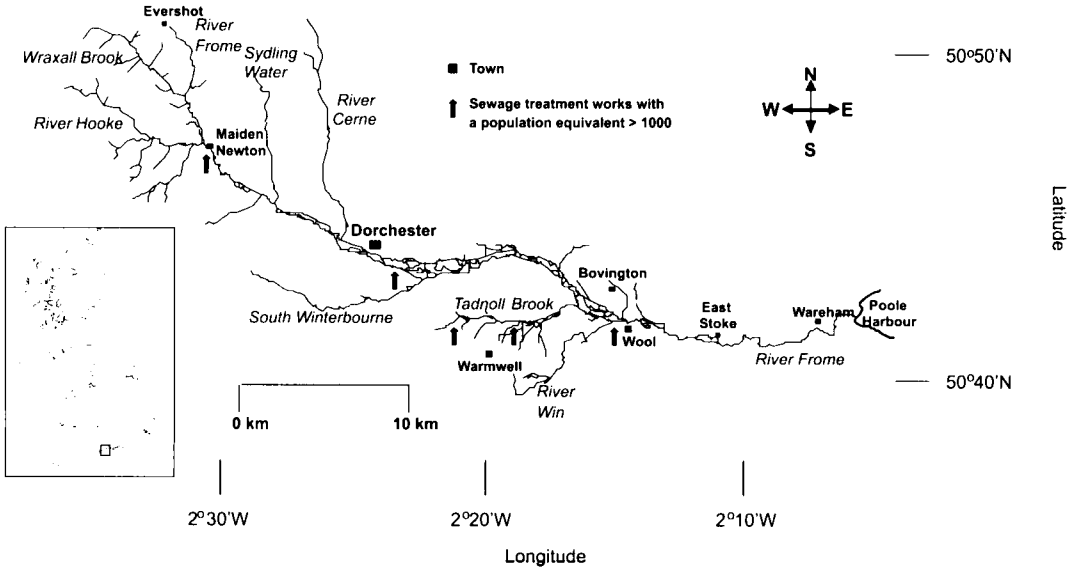
This study was conducted using a series of 11 identical flow-through flumes (12.5 m long × 0.3 m wide). They were constructed from stainless steel and filled with 3 cm of cleaned river substrate consisting mainly of course gravels with some interstitial sands and silts. They were located immediately adjacent to the Mill Stream below the water level of the river. The flumes were supplied with river water, by gravity, directly from the Mill Stream via pipes through the riverbank. The location was unshaded, and all flumes received similar light levels.

Flume experiments

This study consisted of two experiments: the first was conducted between 29 June and 4 July 2005 and the second between 8 and 17 August 2005. Before the commencement of each experiment, the water velocity through each flume was standardized to 0.12 $m \cdot s^{-1}$ by adjusting valves on the water inlet pipe of each flume. The water velocities were measured using a SENSA RC-2 ultrasonic flowmeter. The depth of the water was standardized at 4 cm above the gravel substrate in the middle of each flume by adjusting the height of a weir at the end of the flumes. This gave a water through-flow rate of ~1.4 $L \cdot s^{-1}$. Stone dams were then constructed 2 m downstream from the inlet pipe to produce a deeper (12 cm) section at the front of each flume to increase the residence time of the river water to over 1 min in this impounded upper section. This improved the mixing of the different nutrient treatments before entering the main flume section and allowed time for chemical precipitation reactions to occur for the iron(II) sulphate ($FeSO_4$) treatments described below.

One of three treatments was randomly assigned to each flume. Some flumes had the SRP concentrations of their river water increased by the continuous addition of a concentrated potassium dihydrogen orthophosphate (KH_2PO_4) solution in deionized water dripped directly into the water inflow using a peristaltic pump. A range of SRP concentra-

Fig. 1. Map of the River Frome catchment in Dorset, UK (inset).



tions was achieved by using varying drip rates, with the maximum rate quadrupling the mean SRP concentration observed in the Mill Stream during the experimental period. The mean SRP concentration in the river between June and August 2005 was 120 $\mu g \cdot L^{-1}$, which is classified as meso-eutrophic in the United Kingdom (Mainstone et al. 2000) and exceeds the UK government's 60 μg SRP$\cdot L^{-1}$ target for chalk rivers (Environment Agency 2000). Therefore, a doubling of SRP concentration will exceed the interim target for heavily enriched rivers of 200 $\mu g \cdot L^{-1}$, and an SRP concentration in excess of 400 $\mu g \cdot L^{-1}$ should produce hypereutrophic conditions (Mainstone and Parr 2002).

The SRP concentrations in other flumes were decreased by the addition of a concentrated $FeSO_4$ solution in deionized water dripped directly into the flume inflow. The $FeSO_4$ solution was added either by using a peristaltic pump (for low addition rates) or by siphoning the iron solution from a constant-head stock tank into the flumes and controlling the addition rate using tube clamps (for flumes requiring higher addition rates). The added $FeSO_4$ reacts with dissolved phosphate ions present in the incoming river water, rapidly forming insoluble (and therefore nonbioavailable) $Fe_3(PO_4)_2$ (Reynolds and Davies 2001; Suschika et al. 2001). Dosing with iron salts has been routinely used by the water industry across Europe to decrease soluble phosphate concentrations in wastewater effluents and water inputs to reservoirs (Bernhardt and Clasen 1982; Perkins and Underwood 2001). The dammed front section of the flumes increased the water residence time, allowing the P-stripping reaction to occur before reaching the main algal monitoring section of the flume. The dam also prevented much of the resulting $Fe_3(PO_4)_2$ precipitate from entering the main flume channel. Target SRP concentrations were produced by using different rates of $FeSO_4$ addition, determined during previous pilot studies. The remaining flumes received no additions of KH_2PO_4 or $FeSO_4$ and acted as experimental controls.

Immediately before the start of the experiments, the gravel substrates in all of the flumes were agitated to release any fine sediment and filamentous algae that had accumulated prior to the start of the experiment. Algae that had grown along the sides of the flumes were removed by scrubbing. The flumes were searched for invertebrates and any found were removed. After all of the flumes were cleaned and appeared identical, the planned treatments (P addition, $FeSO_4$ addition, and control) were randomly assigned to the flumes and the additions of KH_2PO_4 and $FeSO_4$ commenced.

Three flumes were used during Experiment 1. One flume received SRP additions (which aimed to double the River Frome SRP concentration from 120 to 240 $\mu g \cdot L^{-1}$), one flume was dosed with $FeSO_4$ (aiming to reduce the River Frome SRP concentration by 80% to between 20 and 30 $\mu g \cdot L^{-1}$), and the remaining flume received no treatment and acted as an experimental control. Experiment 2 used 11 flumes. Two flumes received SRP additions (targeting a doubling and quadrupling of the River Frome SRP concentration), five flumes received a range of $FeSO_4$ dosing, and the remaining four flumes received no additions and acted as experimental controls. After 90 min of P and $FeSO_4$ addition, water samples were taken from the middle of each flume, filtered through a 0.45 μm membrane filter (WCN grade; Whatman, UK), and analysed for SRP concentration (see Water chemistry analysis section below), which confirmed that the range of SRP concentrations was satisfactory and the P stripping was working. A rectangular slate tile (0.52 m × 0.26 m) (previously thoroughly scrubbed and washed in deionized water) was then placed in each flume, 2 m downstream of the dam, to act as clean growth substrates for epilithic algae to colonize.

The SRP concentrations of the flumes were monitored between four and six times per day throughout the course of each experiment. Water samples were taken from the downstream end of the dammed sections to give time for the KH_2PO_4 and $FeSO_4$ treatments to mix and react and to avoid any disturbance of the algae and sediment around the slate tiles during water sampling. A 50 mL sample was taken using a syringe and then immediately filtered through a 0.45 μm membrane filter. Samples were stored at 4 °C in the dark

and analysed for SRP within 12 h to avoid errors associated with sample instability (Haygarth et al. 1995; House and Warwick 1998).

Water flow rates and depths and treatment drip rates were monitored and adjusted at 2 h intervals throughout the day. Plant debris and invertebrates that had been washed into the flumes from the Mill Stream were regularly removed so that they did not disturb or consume the algae growing on the slate tiles.

Water samples (500 mL) were also taken from the Mill Stream upstream of the flume facility during the two experiments to allow the nutrient concentration of the incoming flume water to be monitored. The average sampling frequency was five per day, taken using an automatic water sampler (model 1011; Montec Epic, Manchester, UK). The water sampler inlet pipe was positioned to face downstream at a fixed height above the bed of the river (approximately at the midpoint of the water column) within the main flow of the river. Aliquots of these Mill Stream samples were filtered through a 0.45 μm membrane filter (WCN grade; Whatman, UK) and analysed for SRP and total oxidizable N (TON). The remaining unfiltered samples were analysed for total P (TP) concentration.

The two experiments were run until a strong, visible algal growth had developed on some of the slates. In Experiment 1, slates were immersed for 4 days, and in Experiment 2, the slates were left for a longer period (9 days). At the end of each experiment, the slates were carefully removed and the algal–sediment layer from each slate was transferred into an airtight sampling jar. This was immediately returned to the laboratory, stored in the dark at 4 °C (so as to avoid degradation of the chlorophyll), and analysed for chlorophyll *a* concentration within 24 h (HMSO 1986). Bulk samples of water were then taken from the downstream end of each flume and analysed for pH and suspended solids concentration.

Water chemistry analysis

The filtered water samples were analysed for SRP, TON, and dissolved reactive Si concentration using a SEAL AQ2 discrete multichemistry analyser (Synermed Analytical and Environmental Ltd., Burgess Hill, UK). Each batch of samples was analysed alongside quality control nutrient standards.

SRP concentration was determined using a spectrophotometric method (Murphy and Riley 1962). SRP present in the water sample was reacted with an acid molybdate reagent, in the presence of antimony, to form a yellow-coloured phosphomolydate complex. This was then reduced by the addition of ascorbic acid to form the intensely coloured phosphomolybdenum blue, which was quantified spectrophotometrically at a wavelength of 880 nm. TON was quantified by reducing all nitrate ions present in the sample to nitrite by reaction with hydrazine in alkaline conditions using cupric ions as a catalyst. This reduced nitrate, plus any nitrite present in the original sample, was then reacted with sulphanilamide and *N*-(1-naphthyl)-ethylenediamene dihydrochloride to form red-coloured azo dye, which was quantified spectrophotometrically at a wavelength of 546 nm (SEAL 2004). Dissolved reactive Si concentration was determined by reaction with ammonium molybdate to form molybdosilicic acid, which was then reduced by the addition of ascorbic acid to form a silicomolybdenum blue complex. This was quantified spectrophotometrically at a wavelength of 880 nm (Mullin and Riley 1955).

TP concentration was determined by digesting unfiltered samples with acidified potassium persulphate in an autoclave (121 °C, 40 min). The digest was then reacted with acidified ammonium molybdate to form an intensively blue-coloured molybdenum–P complex, which was quantified spectrophotometrically at a wavelength of 880 nm (Eisenreich et al. 1975).

Suspended solid concentrations were determined by filtering a known weight of previously unfiltered sample through preweighed GF/C-grade glass microfibre filter paper (Whatman, UK) and then oven drying the filter and sediment overnight at 105 °C.

Chlorophyll *a* analysis

The algae biomass on each slate was quantified by chlorophyll *a* analysis. The algae–sediment samples from each slate were diluted to 1 L with deionized water. The jar was shaken vigorously to homogenize the sample and then subsampled in triplicate (20 mL). Each subsample was filtered through a 0.45 μm membrane filter. The chlorophyll *a* on each filter was quantified spectrophotometrically following overnight methanol extraction (Marker 1972; HMSO 1986; Gainswin et al. 2006) using a Beckman DU520 spectrophotometer.

Nutrient-diffusing periphytometers

Nutrient-diffusing periphytometers (Matlock et al. 1998) were used to determine whether high $FeSO_4$ additions to the flumes had a detrimental effect on algal growth. The periphytometers consisted of a 100 mL polyethylene bottle with a 20 mm diameter hole drilled through the cap. The bottles were filled with either deionized water (control) or a concentrated P solution (20 mmol $Na_2HPO_4 \cdot L^{-1}$ in deionized water). A diffusion membrane with a 12 000 to 14 000 Da pore size (Medicell; London, UK) and a glass fibre filter paper (grade MF300; Fisher Scientific, UK) were placed over the neck of the bottle and held in place by the bottlecap. The membrane allowed nutrient to diffuse out of the periphytometer but prevented algae from colonizing the inside of the bottle and thereby depleting the excess nutrient. The filter paper served as a substrate for algal growth.

The periphytometers were deployed during Experiment 1. Five control and five P-enriched periphytometers were placed (in a randomized order) in the downstream end of Flume 1, which was receiving a maximum dose rate of $FeSO_4$. The periphytometers were left in the flume until the end of the experiment (5 days). They were then carefully removed, dismantled, and the mass of algae on each filter quantified by chlorophyll *a* analysis (HMSO 1986).

Nutrient-diffusing periphytometers were also deployed in the River Frome on 16 June 2005. The periphytometers were filled with one of eight nutrient-enrichment treatments: P, Si, N, deionized water (control), P + N, P + Si, N + Si, and all three nutrients. Each nutrient treatment had three replicates. The array of periphytometers was placed inside a floating mesh cage (to decrease the influence of fish and invertebarate grazing and to prevent plant debris covering the bottles) at a depth of 4 cm below the river surface. After 6 days,

the periphytometers were removed, dismantled, and the filter paper substrates analysed for chlorophyll *a* concentration.

Mill Stream monitoring

Mill Stream water samples were taken between February and December 2005 at a minimum frequency of two per day using an Epic water sampler. The sampling rate was increased to as many as eight per day during storm events. All samples were analysed for SRP, TON, and TP concentration. This 10-month data set was gathered to monitor changes in concentration and nutrient ratio throughout the year and allowed the nutrient concentrations observed during the flume experiments to be put in the context of an annual pattern.

Statistical analysis

For all statistical analyses, Ryan–Joiner tests were performed to confirm that the data were normally distributed, and homogeneity of variances was tested using Bartlett's test. Analysis of variance (ANOVA) was used to assess differences in chlorophyll *a* concentration in the P addition, control and $FeSO_4$ addition flumes in Experiment 1 and to determine the effect of different periphytometers nutrient treatments. When ANOVA was significant, differences between treatments were tested using the Tukey multiple comparison test. Coefficients of variance of the SRP concentrations produced in each flume in Experiment 2 were calculated to confirm that the variation in P concentration was relatively similar for each treatment. Data were analysed using Minitab statistical software, release 14 (Minitab Inc., State College, Pennsylvania).

Results and discussion

Experiment 1

Nutrient concentrations

The P addition and precipitation treatments resulted in actual mean SRP concentrations that were within 8% of the target concentrations. The SRP concentrations observed in each flume over the course of Experiment 1 are shown in Fig. 2. Flume 2 received no treatment, and this unaltered River Frome water had an initial SRP concentration of 16 $\mu g \cdot L^{-1}$, gradually decreasing to 118 $\mu g \cdot L^{-1}$ by the end of the experiment (mean concentration = 132 $\mu g \cdot L^{-1}$). These SRP concentrations are similar to the mean values observed in the River Frome in July 1999 and 2000 (143 and 152 $\mu g \cdot L^{-1}$, respectively) (Bowes et al. 2005*b*). The addition of P solution to Flume 3 increased the SRP concentration of the river water to between 213 and 289 $\mu g \cdot L^{-1}$ (mean SRP concentration = 246 $\mu g \cdot L^{-1}$), which was equivalent to a 90% increase in SRP concentration. The addition of $FeSO_4$ to Flume 1 decreased the SRP concentration of the river water to between 21 and 42 $\mu g \cdot L^{-1}$ (mean concentration = 32 $\mu g \cdot L^{-1}$), a reduction in mean SRP concentration of 75%. Analysis of flume water samples confirmed that TON and Si concentrations were the same as those measured in the River Frome, thereby confirming that the $FeSO_4$ additions had no effect on N and Si. The mean TON concentration in the River Frome during this period was 6.0 $mg \cdot L^{-1}$ (Fig. 3), which meant that the three flumes had a mean N:P molar ratio of 415, 101, and 54 for the $FeSO_4$ addition, control, and P addition treatments, respectively. These ratios are much higher than the 16:1 N:P Redfield ratio (Redfield 1958) and so imply that P could be limiting to algal growth owing to the excess of bioavailable N. However, concentrations of both nutrients were relatively high in all flumes, and so the Redfield ratio may not be an effective means of determining nutrient limitation.

Periphyton biomass

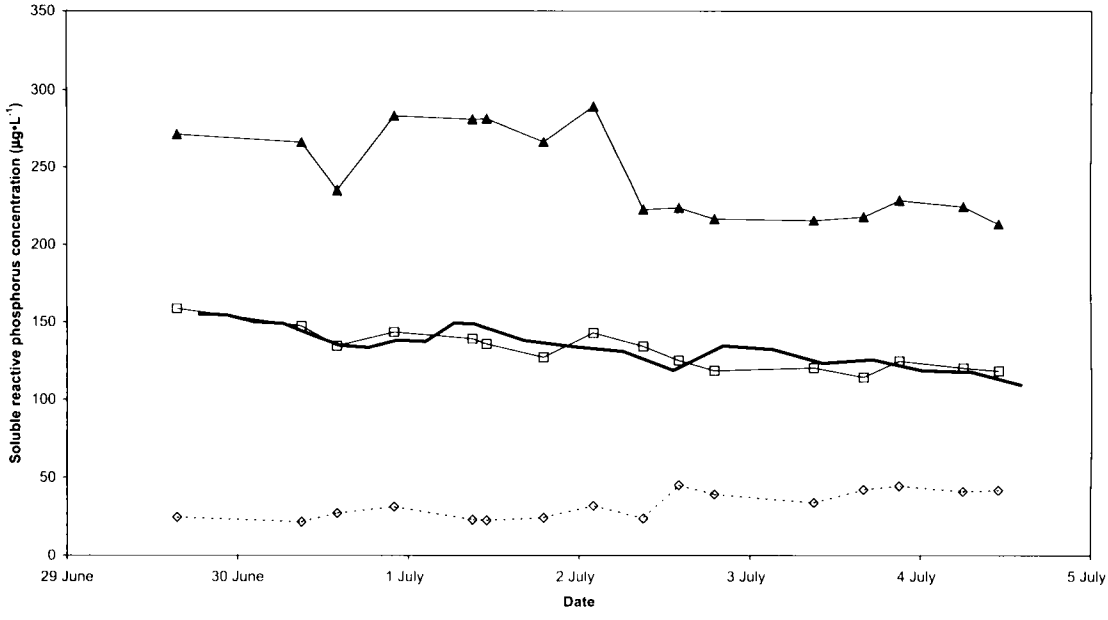
After 4 days of immersion, the slate tiles in all three flumes had been colonized by filamentous algae of up to 5 mm thickness. The experiment was terminated and the slates were removed at this point, as some small-scale sloughing of the biofilm had been observed in the control and P addition flumes. Periphyton biomass was estimated by quantifying the density of chlorophyll *a* on each slate (Fig. 4).

There was no significant difference between the control and P addition flumes, whereas the flume that received the $FeSO_4$ addition had significantly lower chlorophyll *a* concentration (Tukey's test, ANOVA, $F = 50.3$, $p < 0.001$). The mean chlorophyll *a* concentrations of the control and P addition flumes were 9.1 and 9.3 $\mu g \cdot cm^{-2}$, respectively, despite the P addition flume having a mean SRP concentration of 246 $\mu g \cdot L^{-1}$. This result implies that P was not limiting algal growth at concentrations above 130 $\mu g \cdot L^{-1}$. Almost doubling the SRP concentration in the River Frome during the critical summer growing period would therefore be unlikely to have any effect on the rate of periphyton growth or biomass in the lower River Frome; consequently, increases in SRP concentration would not lead to an increase in eutrophication and a decline in environmental water quality. Studies by Jarvie et al. (2002*b*) have observed a similar lack of ecological response to a sustained increase in SRP concentration (up to 160 $\mu g \cdot L^{-1}$) in the River Kennet, UK.

The flume that received the $FeSO_4$ addition had significantly lower chlorophyll *a* concentration (5.2 $\mu g \cdot cm^{-2}$) (Fig. 4). This 44% decrease in chlorophyll *a* density on the slate substrates implies that the reduced concentration of bioavailable P in the overlying water is either reducing the growth rate of the periphyton or reducing the biomass of algae that can be sustained under these potentially P-limited conditions. Therefore, a similar reduction in SRP concentration in the River Frome to 32 $\mu g \cdot L^{-1}$ during this monitoring period would cause a significant reduction in periphyton growth rate and would result in a significant improvement in water quality of the river. These results agree with the eutrophication classification system devised by Mainstone and Parr (2002) derived from SRP concentrations and N:P ratios of 5000 river monitoring sites across England and Wales. They identified 50 μg SRP$\cdot L^{-1}$ as being the concentration below which P is likely to become limiting throughout the year. Based on this classification, the control and P addition flumes were only deemed to be possibly P limited for part of the growing season, whereas the P-stripped flume would be classified as being likely to be P limited.

However, the reduced periphyton colonization in the P-stripped flume could also be due to high Fe concentrations somehow inhibiting algal growth or other physical effects of $FeSO_4$ addition, such as changes in river water pH (Perkins and Underwood 2001). The pH values of the flumes were all

Fig. 2. SRP concentrations during Experiment 1. ◇, $FeSO_4$ addition (Flume 1); □, control (Flume 2); ▲, P addition (Flume 3). SRP concentrations measured in the River Frome are represented by the thick solid line.



7.7 (measured just before the end of Experiment 1), and so the reduced algal growth on the slates was not due to changes in pH caused by $FeSO_4$ addition. The reaction of $FeSO_4$ with dissolved P results in the formation of a precipitate, and this may increase the turbidity of the overlying river water (although most of the precipitate was retained in the upper section of the flume upstream of the dam). Analysis of bulk water samples taken at the end of Experiment 1 were analysed for suspended solid concentration and confirmed that Flume 1 ($FeSO_4$ addition) had a higher suspended solids concentration (13 $mg \cdot L^{-1}$) than the control (5.3 $mg \cdot L^{-1}$) and P addition flumes (5.9 $mg \cdot L^{-1}$). Therefore, the $FeSO_4$ addition could be "shading out" the periphyton, resulting in less chlorophyll *a* concentration on the Flume 1 slate substrate.

Periphytometers

Control and P-diffusing periphytometers were deployed in Flume 1 to investigate the effects of $FeSO_4$ additions on algal growth. The periphytometer chlorophyll *a* concentration results from this $FeSO_4$-treated flume are provided in Fig. 5*a*. The control periphytometers (no nutrient addition) had a mean chlorophyll *a* concentration of 3.0 $\mu g \cdot cm^{-2}$. The P-diffusing periphytometers had significantly higher chlorophyll *a* concentrations (5.3 $\mu g \cdot cm^{-2}$) (ANOVA, $F = 36.8$, $p = < 0.001$), demonstrating that the periphyton was clearly P limited at an ambient SRP concentration of 32 $\mu g \cdot L^{-1}$. These results suggest that the reduced chlorophyll *a* concentration on the slate substrate in Flume 1 was due to the decreased concentration of SRP in the overlying water and not due to elevated $FeSO_4$ concentration inhibiting algal growth,. This conclusion would have been strengthened if periphytometers had also been deployed in the control and P addition flumes. The authors plan to do this in future studies.

The periphytometer deployment in the River Frome itself, prior to the start of Experiment 1, demonstrated that there was no significant difference in chlorophyll *a* concentration between any of the treatments (Tukey's test, ANOVA, $F = 0.18$, $p = 0.986$) (Fig. 5*b*). These results confirm that algal growth was not P, N, or Si limited (or colimited) during the 16–22 June 2005 deployment period (mean TP, SRP, TON, and Si concentrations = 167 $\mu g \cdot L^{-1}$, 114 $\mu g \cdot L^{-1}$, 5.6 $mg \cdot L^{-1}$, and 2.3 $mg \cdot L^{-1}$, respectively).

Experiment 2

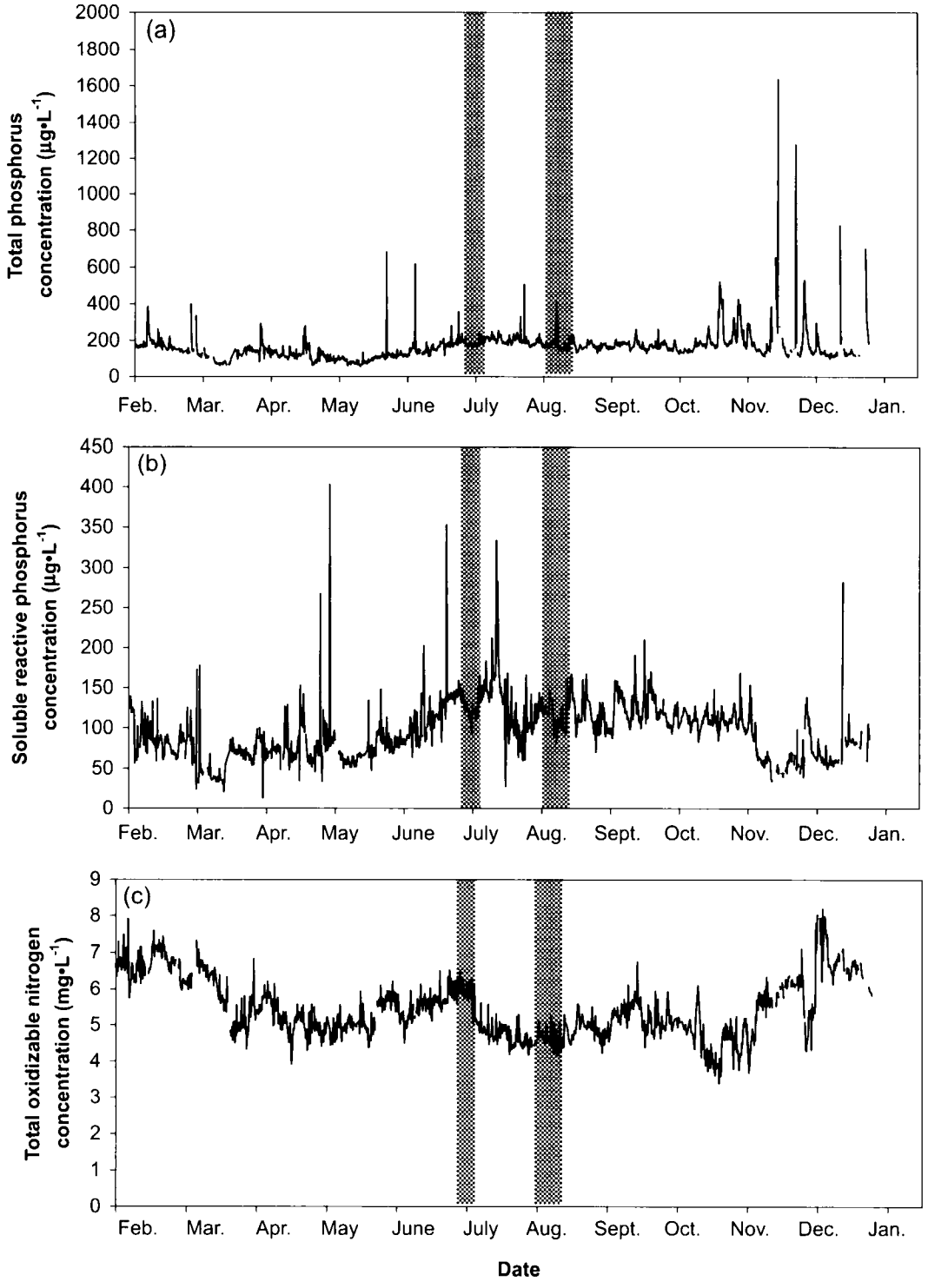
Experiment 1 demonstrated that a wide range of SRP concentrations could be produced and maintained in the flumes over a period of days and that the P-stripping methodology was capable of reducing SRP concentrations to 21 $\mu g \cdot L^{-1}$. The results from the periphytometers deployed in the P-stripped flume confirmed that P concentration was limiting periphyton growth in the River Frome at 32 $\mu g \cdot L^{-1}$. The chlorophyll *a* concentration results in Experiment 1 implied that increases in River Frome P concentration would not produce enhanced periphyton growth, but this was only based on a single observation. Therefore, Experiment 2 aimed to verify this by investigating the effect that a doubling and quadrupling of the SRP concentration in the River Frome would have on epilithic algal growth.

The chlorophyll *a* results from the control and P-stripped flumes in Experiment 1 indicated that SRP concentration begins to limit periphyton growth between 32 and 132 $\mu g \cdot L^{-1}$, but there were clearly not enough data points to determine where the "breakpoint" was (i.e., the P-limiting concentration, where increases in SRP do not lead to increased periphyton growth and reductions in P concentration lead to reductions in growth). To determine this P-limiting concentration, five flumes received different rates of $FeSO_4$ additions so that a wide range of SRP concentrations were produced between the ambient River Frome P concentration and 30 $\mu g \cdot L^{-1}$.

Nutrient concentrations

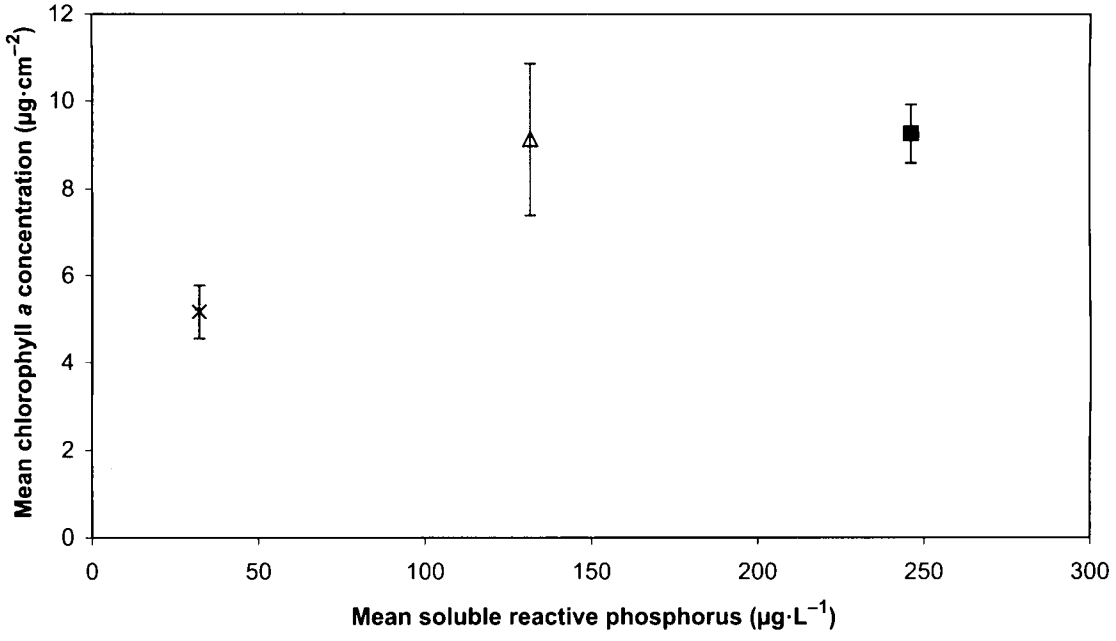
The SRP concentrations observed over the course of Experiment 2 (8–17 August 2005) are shown in Fig. 6. The SRP concentration in the four control flumes (receiving unaltered

Fig. 3. Nutrient concentrations in the River Frome at East Stoke, Dorset, 2005 (shaded areas denote the periods of the flume experiments).



River Frome water) varied between 80 and 150 µg·L^{-1} over the course of the experiment, with a mean SRP concentration of 109 µg·L^{-1}. The mean SRP concentration measured in the River Frome during this period was 111 µg·L^{-1}. These SRP concentrations and fluctuations were typical of the River Frome nutrient chemistry over the July–October 2005 period (Fig. 3), although they were much lower than observed in previous studies (mean SRP concentrations in August 1999 and August 2000 of 176 and 218 µg·L^{-1}, respectively) (Bowes et al. 2005*b*). The two flumes receiving P additions (Flumes 3 and 10) had their mean SRP concentrations increased to 196 µg·L^{-1} (80% increase) and 423 µg·L^{-1} (290% increase), respectively. The coefficients of variance in the P addition flumes (Flume 3 = 17%, Flume 10 = 14%) were very similar to those of the four control flumes (coefficients of variation of between 11% and 14%). The remain-

Fig. 4. Chlorophyll *a* concentrations on slate substrates at the end of Experiment 1. ×, $FeSO_4$ addition (Flume 1); △, control (Flume 2); ■, P addition (Flume 3). Error bars are ±2 SD derived from the three subsamples analysed from each slate. The curve represents the best fit of the data to Michaelis–Menten hyperbolic regression analysis (Dawes 1972).



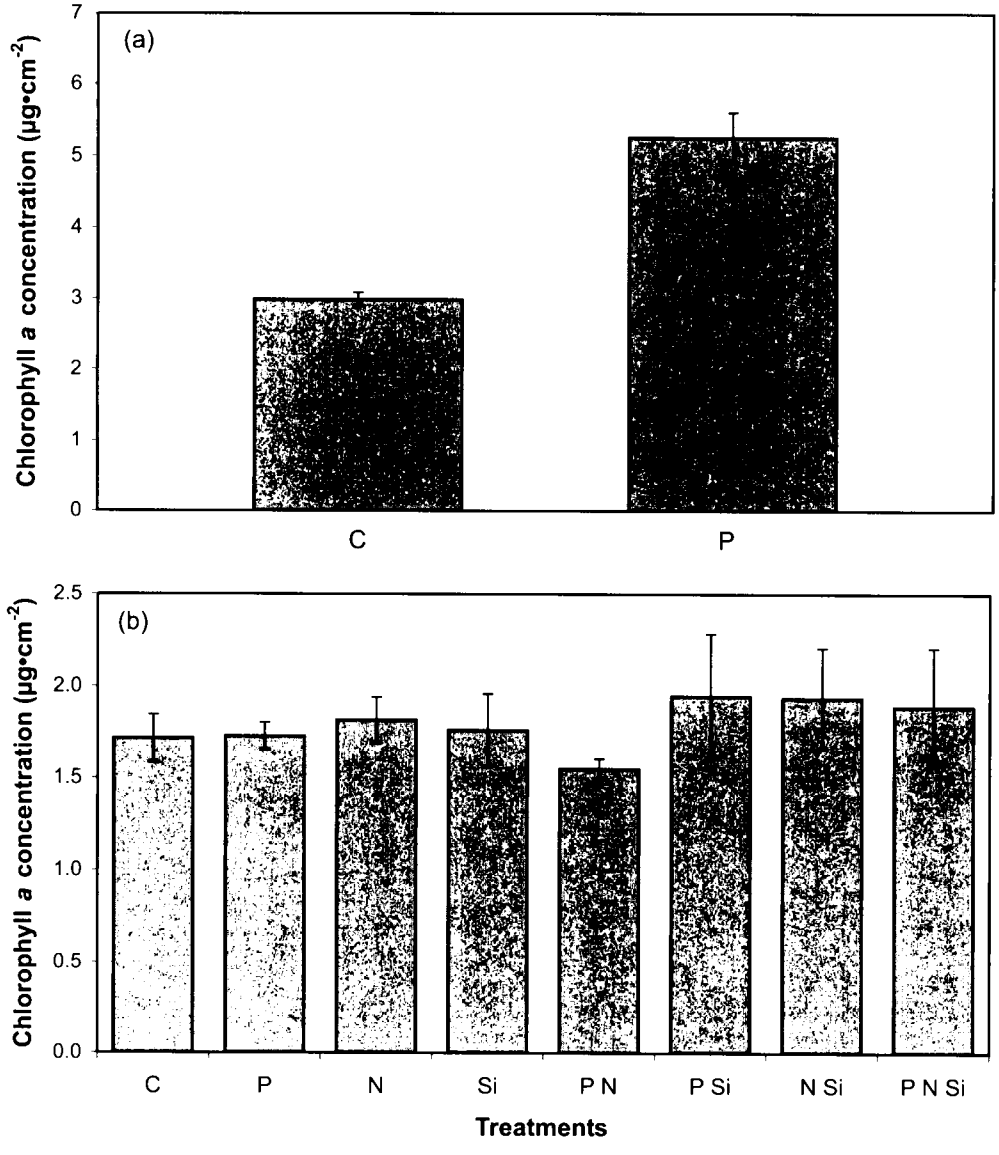
ing five flumes received a range of $FeSO_4$ addition rates, resulting in mean SRP concentrations from 88 µg·L^{-1} (equivalent to a 20% reduction in ambient concentration) to 36 µg·L^{-1} (a 67% reduction). The mean TON concentration in the River Frome during this period was 4.6 mg·L^{-1} (Fig. 3), which meant that the flumes in Experiment 2 had mean N:P molar ratios ranging from 285:1 (for the flume with the highest P-stripping rate) to 24:1 for Flume 10 (receiving the maximum P addition).

Periphyton biomass

After 9 days of the experiment, all slates had been colonized by significant quantities of epilithic algae. Algal sloughing was observed on the P-addition and control slates, indicating that periphyton biomass was approaching its maximum, and so the experiment was terminated. Periphyton biomass was estimated by quantifying the density of chlorophyll *a* on each slate (Fig. 7). Algal biomass showed the same response to increasing SRP concentration that was suggested by the results from Experiment 1. The highest mean chlorophyll *a* concentrations were observed in the four control flumes (between 35.6 and 36.6 µg·cm^{-2}). There was very little variation in the chlorophyll *a* concentrations in these four control flumes, which indicates that there is minimal physical variation between the flumes and that the precision of the sampling and analytical technique is high. The chlorophyll *a* concentrations in the control and P addition flumes were much higher than those observed in other longer-duration studies (Mundie et al. 1991; Tank and Dodds 2003; Sabater et al. 2005), showing that algal growth rates were higher in this nutrient-rich river. Chlorophyll *a* concentrations were higher than those observed in Experiment 1 owing to the 5-day extra duration of Experiment 2, allowing for greater algal accrual. An 80% increase in SRP concentration produced the next highest algal biomass with a mean concentration of 34 µg·cm^{-2}. A further increase in SRP concentration to 423 µg·L^{-1} resulted in a reduction in chlorophyll *a* concentration to 28.4 µg·cm^{-2}. This reduction in algal biomass at elevated SRP concentration may be due to the colonizing algae being increasingly filamentous and friable, which appeared to result in a higher sloughing rate. These results imply that a significant increase in SRP concentration in the River Frome would not have increased the epilithic algal biomass during this study period and indicate that the river was not P-limited.

The five flumes receiving $FeSO_4$ additions all had reduced epilithic algal biomass compared with the control flumes. The flumes with SRP concentrations of 88 and 55 µg·L^{-1} had mean chlorophyll *a* concentrations of 27.3 and 29.8 µg·cm^{-2}, respectively. The three flumes with the lowest SRP concentrations (35.8, 36.2, and 38.9 µg·L^{-1}) had the lowest observed mean chlorophyll *a* concentrations (19.6, 21.4, and 12.2, respectively). This step-change in algal biomass under 50 µg SRP·L^{-1} concentration supports findings from other UK river studies (Mainstone and Parr 2002). Similar patterns of increasing algal biomass with increasing P concentration followed by a leveling off as the P concentration exceeds the maximum P-limiting concentration) have been observed in previous studies (Dodds et al. 1997; Scrimgeour and Chambers 1997; Rier and Stevenson 2006). This pattern is likely to be observed in all rivers, although the breakpoint in the graph will vary depending on the concentration of N and other plant nutrients. In the River Frome, periphytometer studies showed that P, N, and Si were not limiting (or colimiting) algal growth, and therefore, when P concentrations are above the maximum P-limiting concentration of ~90 µg·L^{-1} observed in Experiment 2, epilithic algal growth is likely to be either light limited or growing at its maximum possible rate. This 90 µg SRP·L^{-1} target value is likely to be similar for other comparable nutrient-impacted rivers, but

Fig. 5. Chlorophyll *a* concentrations on nutrient-diffusing periphytometers (*a*) after 5 days of immersion in Flume 1 ($FeSO_4$ addition, mean SRP concentration = 32 $\mu g \cdot L^{-1}$) (n = 5) and (*b*) after immersion in the River Frome, 16–22 June 2005 (n = 3). Error bars are ±1 SE). C, control; P, P addition; N, N addition; Si, Si addition.



this needs to be confirmed by applying this methodology to other catchments.

The pattern shown in Fig. 7 explains why step-reductions in P loading often produce no ecological response in many urbanized lowland rivers (Jarvie et al. 2002*a*), as they have SRP concentrations far in excess of the maximum P-limiting concentration. The P concentration of the river, following remediation, remains above the maximum P-limiting concentration, and so the river remains on the horizontal part of the curve. There is therefore no algal biomass response to changing SRP concentrations.

Phosphorus limitation in the River Frome

The results from both flume experiments show that epilithic algal growth in the River Frome was not P limited throughout the summer of 2005. An increase in SRP concentration during this period would not produce increased algal biomass in the river and so would not lead to an increased level of eutrophication or a decline in environmental water quality. The highest measured SRP concentration in the River Frome in 2005 was a sharp peak of 404 $\mu g \cdot L^{-1}$ (Fig. 3), associated with a rainfall event in May 2005, but Experiment 2 has shown that even an increase in mean SRP concentration to over 420 $\mu g \cdot L^{-1}$ for a sustained period of 9 days did not result in an increase in epilithic algal biomass. These findings are supported by the results from the nutrient-diffusing periphytometers deployed in the River Frome in June 2005, which showed that algae were not limited (or colimited) by SRP, TON, or Si concentration.

The results from the P-stripping flumes indicate that below ~90 $\mu g \cdot L^{-1}$ SRP, algal biomass in the River Frome would decline with decreasing SRP concentration. An SRP concentration of 60–80 $\mu g \cdot L^{-1}$ may cause the epilithic algal biomass to fall by 20%–25%. An SRP concentration of

Fig. 6. SRP concentrations during Experiment 2. Solid lines and symbols, flumes receiving P additions; solid lines and open symbols, control flumes; broken lines and open symbols, flumes receiving $FeSO_4$ additions; thick solid line, SRP concentrations measured in the River Frome.

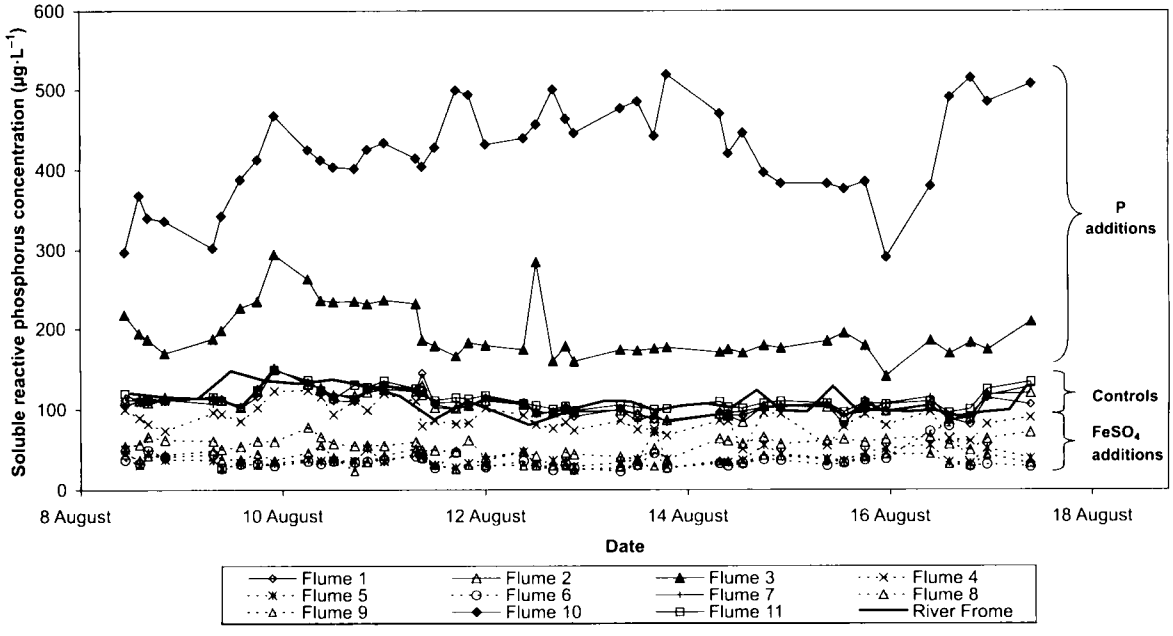
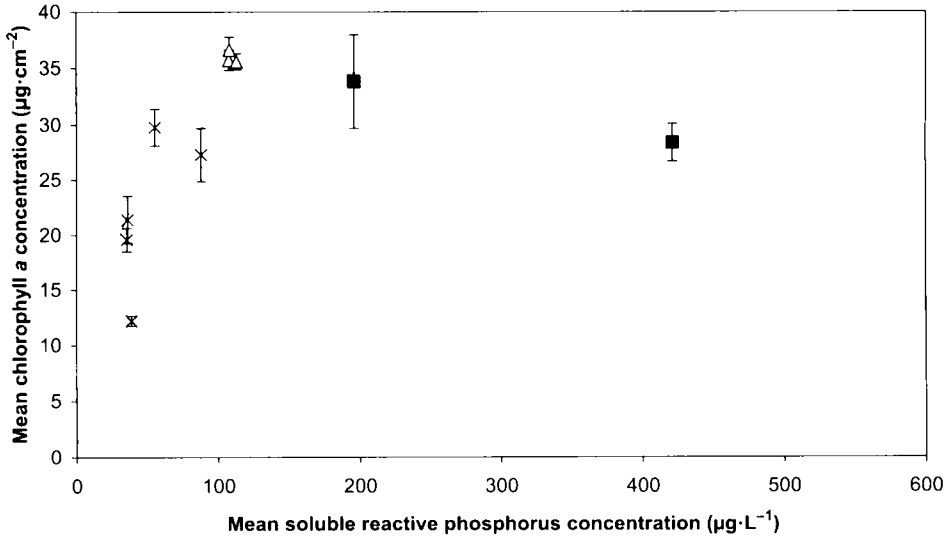


Fig. 7. Mean chlorophyll *a* concentrations on slate substrates at the end of Experiment 2. ×, $FeSO_4$ addition; △, control; ■, P addition. Error bars are ±2 SD derived from the three subsamples analysed from each slate. The curve represents the best fit of the data to Michaelis–Menten hyperbolic regression analysis (Dawes 1972).



<40 µg·L^{-1} may result in a significant (up to 60%) reduction in algal biomass to between 12 and 24 µg chlorophyll *a*·cm^{-2}. The use of nutrient-diffusing periphytometers in Experiment 1 confirmed that this was caused by P limitation at these low SRP concentrations. A desk study by Dodds et al. (1997) suggested a similar target P concentration (30 µg·L^{-1}) to keep epiphytic chlorophyll *a* concentrations below 10 µg·cm^{-2}.

The River Frome intensive monitoring data for 2005 (Fig. 3) shows that the SRP concentration rarely fell below 40 µg·L^{-1} for a sustained period (>12 h), occurring only in March and November. These periods account for only 3% of the monitoring period and occur outside the spring–summer algal growing season typical of rivers in southern England. Therefore, they are likely to have little effect on controlling the total algal biomass in the River Frome over an annual cycle. However, the SRP concentration of the River Frome is between 60 and 90 µg·L^{-1} for much of the annual cycle (Fig. 3), particularly during long periods in March, May, and November 2005, and algal growth would be expected to be suppressed during these periods owing to P limitation. During the critical June–September summer period, when eutrophic events are most likely to occur, the River Frome SRP concentration is at its highest owing to the lack of dilu-

tion of sewage effluent inputs during this low river flow period. The mean SRP concentration in the River Frome at this time is 120 $\mu g \cdot L^{-1}$ and rarely falls below 90 $\mu g \cdot L^{-1}$, and so, algal biomass is likely to be at its maximum. Summer P concentrations need to be reduced until the maximum SRP concentration is below the ~90 $\mu g \cdot L^{-1}$ maximum P-limiting concentration before significant improvements in the environmental status of the River Frome would be observed. This maximum target SRP concentration agrees closely with the UK Environment Agency's mean P concentration target of 60 $\mu g \cdot L^{-1}$ for chalk rivers (Mainstone and Parr 2002).

The use of $FeSO_4$ to reduce bioavailable P concentration in river water provides a convenient method to greatly extend the scope of nutrient-enrichment experiments. Such experiments have previously only been able to simulate the effect of nutrient increases on an aquatic ecosystem. The use of streamside flumes, receiving either P or $FeSO_4$ additions, allows the effects of increasing and decreasing bioavailable P concentration to be simultaneously studied for the first time. $FeSO_4$ P stripping also allows such experiments to be used on rivers that already have P concentrations in excess of that needed for maximum algal growth. It is these highly nutrient-impacted rivers, common across most of the urbanized lowland catchments of the European Union and elsewhere, that are currently the focus of nutrient mitigation.

Determining the SRP concentration at which algae become growth limited is vital for effective eutrophication management, as this provides the basis for nutrient target setting within a catchment. Previous studies have shown that this target SRP concentration varies greatly between study areas, ranging from 3 $\mu g \cdot L^{-1}$ (Scrimgeour and Chambers 1997) to >110 $\mu g \cdot L^{-1}$ (Matlock et al. 1999). As the cost of introducing nutrient remediation measures to a catchment is closely dependent on the P target concentration that is set, it is vital that target setting is "knowledge based" for individual catchments. The new methodology presented in this paper could greatly assist catchment managers and policy makers in setting realistic P targets for individual nutrient-impacted rivers, allow them to identify what specific P load reduction is required before an environmental improvement will be observed, and should quantify reductions in algal biomass that would result from various levels of remediation.

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