The fractionation of phosphorus in UK chalk stream surface waters and its relevance to the regulation and management of water quality

P. J. Shaw[1], K.-C. Leung[1] & D. Clarke[2]
[1]School of Geography and Environmental Science, Faculty of Environmental and Life Sciences, University of Southampton, Highfield, Southampton, SO17 1BJ, UK.
[2] School of Engineering, Faculty of Engineering & Physical Sciences, University of Southampton, Southampton Boldrewood Innovation Campus, Burgess Road, Southampton, SO16 7QF, UK.

Corresponding author
Email: P.J. Shaw@soton.ac.uk
Postal address: School of Geography and Environmental Science, Shackleton Building, University of Southampton, Highfield, Southampton, SO17 1BJ, UK.

Abstract

The regulatory management of river water quality requires measurements of phosphorus that are operationally viable and meaningful in terms of insight into its effects. This need is a particular concern in globally rare and ecologically sensitive chalk streams. P data pertaining to rivers are commonly limited to soluble reactive P; other fractions of P may be of concern but are not routinely monitored. This study seeks to establish the nature and extent of non-regulated forms of P in UK chalk streams. Whilst soluble reactive P in two southern English chalk streams was found to comprise the majority of reactive P in surface waters in the majority of samples, 15-20% of the total reactive P was within other size fractions greater than 0.22 µm. The contribution of reactive P to the total P was highly variable. We conclude that, with some adjustments, the established method of regulatory monitoring of P in UK rivers is viable and valuable. In cases where the levels of reactive P are not consistent with ecological status and/or expected outcomes of programmes of measures, we recommend that targeted analysis of non-regulated forms of P is undertaken as a means to guide and focus management interventions.

Keywords

Chalk streams, Phosphorus, Fractionation, Regulation, Management.

1. Introduction

1.1 Nutrients in rivers: problems and causes

Nutrients are critical in determining the ecology of rivers (Hilton *et al.*, 2006; Mainstone, 2010). Natural nutrient inputs to rivers are augmented by anthropogenic sources and activities, leading to their elevation (Blaas & Kroeze, 2016; Mainstone, 2010; Meybeck, 1982). In freshwaters, P is of concern due to its role as a limiting factor for autotrophic organisms (Mainstone & Parr, 2002; Schindler, 1977; Vollenweider, 1968). Anthropogenic P sources include sewage-related inputs (e.g. Richards *et al.*, 2015; Wood *et al.*, 2005;), agriculture (e.g. Daniel *et al.*, 1998; Haygarth *et al.*, 1998; Heathwaite *et al.*, 2006; Kleinman *et al.*, 2015) and industrial wastewaters (e.g. Bowes *et al.*, 2008; Jarvie *et al.*, 1998).

Anthropogenic elevation of P is associated with deterioration of lotic ecosystems (Conley *et al.*, 2009; Schindler, 1977), notably of their flora (Carvalho & Moss, 1995). P from the surrounding landscape and activities therein increases the potential for plant growth (Haygarth *et al.*, 2005). Elevated P inputs typically cause increased plant biomass, a shift towards nutrient-tolerant species, and a switch to an algal-dominated plant community, with undesirable consequences for ecosystem and function (Mainstone 1999), and for the value of rivers to humans.

1.2 Forms and interactions of phosphorus in freshwater

P in freshwater exists in many forms, movement between which is dynamic and complex (Withers & Jarvie, 2008). Physical forms include soluble and colloidal fractions in suspension (Jones *et al.*, 1993; Shaw *et al.*, 1992, 1996, 2000), and particulate forms with which P may be surface-associated or an integral component (Shaw, 1994). Chemical forms include inorganic (Fox, 1993), organic (Whitton & Neal, 2011; Yates *et al.*, 2016) and combinations thereof (Shaw, 1994; Shaw *et al.*, 2000). Interactions of P with river biota are strongly influenced by light and water flow (Hilton *et al.*, 2006), and via direct and indirect mechanisms (O'Hare *et al.*, 2018).

1.3 Regulation of phosphorus in rivers

1.3.1 Overview

Measures to ameliorate impacts of elevated P on rivers employ regulatory frameworks with thresholds for concentrations of specified type(s) of P (JNCC, 2016; UKTAG, 2012, 2013, 2014). Evidence underpinning regulation draws on large-scale and long-term data (Mainstone, 2010). Ranges and thresholds for P align with biological status, aiming to match the biological classification (Mainstone, 2010; UKTAG, 2013, 2014).

The relationship between P and the biological status of rivers is equivocal. In the UK, failures to meet biological targets are more frequent than for P targets, which have been revised due to inconsistent outcomes for ecological status derived using biological and P measures (UKTAG, 2013). Failures to achieve targets for river quality in the UK have nonetheless been attributed to elevated nutrient concentrations (Carvalho & Moss, 1995; UKTAG, 2013); mitigation of P in rivers does not guarantee improved ecological status (Demars *et al.*, 2005; Jarvie *et al.*, 2013).

1.3.2 Regulatory measurements of phosphorus in rivers

Understanding interactions between forms of P (Fox, 1993; Withers & Jarvie, 2008) and riverine biota requires extensive measurements to determine within-river P dynamics (Jarvie *et al.*, 2002). However, assessments for regulatory purposes should ideally be simple, rapid and inexpensive (Jarvie *et al.*, 2013). Established analyses orientate towards chemical species of similar type (Burton, 1973) and applied on a pragmatic basis (Jarvie *et al.*, 2002). Fractions of P are operationally-defined, including removal of particulate materials through filtration or settlement (JNCC, 2016; UKTAG, 2012, 2013, 2014).

Criteria for regulatory assessment of P are in practice relatively simple (Jarvie *et al.*, 2013). In the UK “reactive P” (RP) is defined as molybdate-reactive P (Burton, 1973; Murphy & Riley, 1962) in water samples after the settlement of particulate materials (e.g. UKTAG, 2013, 2014). RP is customarily recorded as “ortho-P” by the Environment Agency (EA) in England; samples may be filtered in the field as well as settled prior to analysis (EA pers. comm.). RP is considered highly similar to soluble reactive P (SRP) in filtered samples (UKTAG, 2013) and is assumed to exclude P associated with particles and/or not readily hydrolysable. Annual mean RP concentration is usually based on monthly samples (UKTAG, 2013, 2014). SRP is an operationally-defined fraction of P and cannot be assumed to comprise orthophosphate, i.e. a chemically-defined species containing P (Jarvie *et al.*, 2002), or comprise entirely inorganic P.

Whilst serving regulatory monitoring needs (Jarvie *et al.*, 2002), RP is an imperfect measure in terms of its biological meaning (Dorich *et al.*, 1985). The reagents used in colourimetric analysis (Burton, 1973; Murphy & Riley, 1962) can lead to over-estimation of inorganic P concentrations (Ballantine *et al.*, 2009). The capacity of riverine flora to assimilate forms of P is critical (Boström *et al.*, 1988; Mainstone & Parr, 2002; Whitton & Neale, 2011); RP is considered to be readily available to biota, but “reactive P” and “bioavailable P” are not synonymous (Boström *et al.*, 1988).

1.3.3 Non-regulated forms of phosphorus in rivers

Monitoring of RP offers some insight to P in a river ecosystem (Jarvie *et al.*, 2002). P exists in inorganic, organic, soluble, particulate and colloidal forms, bioavailability of which is highly variable (Fox, 1993; Hanrahan *et al.*, 2005; Withers & Jarvie, 2008). Removal, release and transformation of P within river systems (Ballantine *et al.*, 2009; Evans & Johnes, 2004; Evans *et al.*, 2004) are influenced by the settlement and resuspension of particulates (House, 2003). Settlement of particulate materials can sequester soluble P from the overlying water (Mainstone *et al.*, 2000) that may be exchangeable (House, 2003). Storage and release of particle-associated P (Evans & Johnes, 2004) strongly influence the availability of P in rivers. Sediment-associated P can lead to in-river storage (Ballantine *et al.*, 2009; Evans & Johnes, 2004; House, 2003; Wang and Li, 2010) and influence downstream transport of P (Demars *et al.*, 2005). Interactions are complex: particulate materials are spatially and temporally variable (Bowes *et al.*, 2005; Evans *et al.*, 2004), and mechanisms are influenced by numerous factors (Fox, 1993).

Forms of P that are not molybdate-reactive may be available to biota. Soluble organic P available to biota (Whitton & Neale, 2011) would only be readily detectable using the molybdenum blue method after oxidative digestion of samples to transform organic P to hydrolysable inorganic form. RP measures may thus underestimate bioavailable P.

1.4 Phosphorus in chalk streams

1.4.1 Chalk streams: overview

Despite efforts to reduce P levels in rivers (Mainstone & Parr, 2002; Sharpley *et al.*, 2013; Withers & Lord, 2010), problems remain, notably in chalk streams. Chalk streams are globally rare (EA & EN, 2004), occurring only in England, France, Belgium and New Zealand (HBP, 2000); most are located in England (WWF, 2014). Chalk streams are notable for their groundwater contribution to flow (Holman *et al.*, 2010; Stuart & Lapworth, 2016), which typically accounts for around 90% of the annual discharge (Mainstone, 1999). The baseflow index for English chalk streams is variable: ranging, for example, from 0.53 to 0.98 (Johnes, 2007). The high baseflow leads to relatively stable temperature (Berrie, 1999) and hydrology (Sear *et al.*, 1999).

England's chalk streams are designated under European regulatory frameworks (HBP, 2000; NE & EA, 2004) as Special Areas for Conservation (SAC; Directive 92/43/EEC; EU, 1992) and Special Protection Areas (Directive 79/409/EEC; EU, 1979), and as salmonid fisheries (Directive 78/659/EEC; EU, 1978). Conservation designations include Sites of Importance for Nature Conservation and Sites of Special Scientific Interest (SSSI; HBP, 2000). Chalk streams are considered sensitive to deterioration. So-called "Chalk stream malaise" comprises loss of macrophytes (notably *Ranunculus* spp.; Mainstone, 1999), excessive benthic and filamentous algae, low water clarity, and siltation of river beds (Jarvie *et al.*, 2006). The gravel-dominated substrate of chalk streams (Sear *et al.*, 1999) is also sensitive to siltation impacts on the reproductive success of salmonid fish (Acornley & Sear, 1999).

1.4.2 Phosphorus in chalk streams and its regulation

Availability of P to biota is critical to chalk stream ecosystem structure and function (Mainstone & Parr, 2002). This P system is both complex and dynamic (House, 2003; Withers & Jarvie, 2008); P exists in different forms across a spectrum of sizes and with differing availability to the flora, knowledge of which provides insight to the form and function of riverine P (Withers & Jarvie, 2008). Such insight is valuable due to the anthropogenic nutrient sources present (HBP, 2000; Mainstone, 1999). Sources include agriculture (livestock, arable, fish, and watercress farms), sewage-related discharges (unsewered and sewered), and terrestrial run-off (HBP, 2000; Mainstone, 1999). The contribution of groundwater to surface streams (Mainstone, 1999; Johnes, 2007; Holman *et al.*, 2010; Stuart & Lapworth, 2016) adds further to the complexity of P in chalk streams.

Regulatory frameworks (§1.3.2) offer some, if limited, insight to the impacts of P upon a chalk river. To provide greater insight by extending the analytical scope of P determinants (§1.3.3) has resource implications. Regulation requires data that are sufficiently meaningful for their intended application: there are risks that (1) increased rigour in sampling and analysis incurs an unnecessary burden upon regulators, and (2) insufficient rigour may be inadequate to underpin programmes of measures for pollution prevention. Methods that are of necessary and sufficient rigour are therefore critical to the successful regulation of P, particularly in internationally-rare chalk streams (EN & EA, 2004; WWF, 2014).

1.5 Aims of the study

The first aim was to elucidate the relative contributions of different fractions of P to overall P in chalk streams, differentiated by size and reactivity. The second aim was to determine whether and how the inclusion of different P fractions influences the appraisal of water quality status in relation to P concentrations. We also consider the implications of P fractionation for the regulatory management of chalk streams.

2. Materials and methods

Sample collection and analyses were designed to represent P in chalk streams at different times of the year with extensive spatial coverage of two upper catchments. Two neighbouring catchments in southern England, the Rivers Itchen and Test, were sampled at multiple locations on the same day, ensuring collection under similar prevailing environmental conditions. Analysis of samples quantified P fractions in surface waters on the basis of (1) reactivity (§2.3.1), and (2) size (§2.3.2).

2.1 Site descriptions

2.1.1 The River Itchen

The Itchen (Fig. 1) is a highly-regarded chalk stream (EA, 2006a), a SSSI and a SAC (The Wildlife Trusts, 2015). It provides spawning sites for Atlantic salmon (*Salmo salar*, L.) and supports juveniles before their seaward migration. The Itchen and its catchment provide recreational angling, agriculture (including watercress and fish farming), public water supply and sewerage services, industry, irrigation, and amenity and recreation (EA, 2006a).

The Itchen rises on chalk geology as three spring-fed tributaries (Fig. 1), flowing into Southampton Water on the south English coast (EA, 2006a). The upper Itchen is 45km long (EA, 2006a) and its surface area is 236.8 km^2. It is entirely underlain by chalk; land use comprises arable/horticultural farming (50%), grassland (32%), woodland (12%) and scattered urban areas (4%) (CEH, 2020a). Long-term average annual rainfall for the upper Itchen (1961 to 1990) was 848 mm y^{-1} (CEH, 2020a).

2.1.2 The River Test

Rising in an area underlain by chalk, the Test (Fig. 1) is 64 km long and is a SSSI along its whole length (EN & EA, 2004), excluding its tributaries. Average long-term annual rainfall from 1941 to 1990 in the catchment was 834 mm y^{-1} (CEH, 2020b). The middle and upper Test are highly regarded for dry fly fishing for salmonids (EA, 2006b). The Test provides for agriculture (including watercress and fish farming), public water supply and sewerage services, industry, irrigation, amenity and recreation. The upper catchment of the Test covers an area of 453 km^2 of which 99.8% is underlain by chalk bedrock (CEH, 2020b). Land use in the upper Test catchment comprises arable farming and horticulture (50.4%), grassland (29%), woodland (14%) and some urban settlements (3%) (CEH, 2020b).

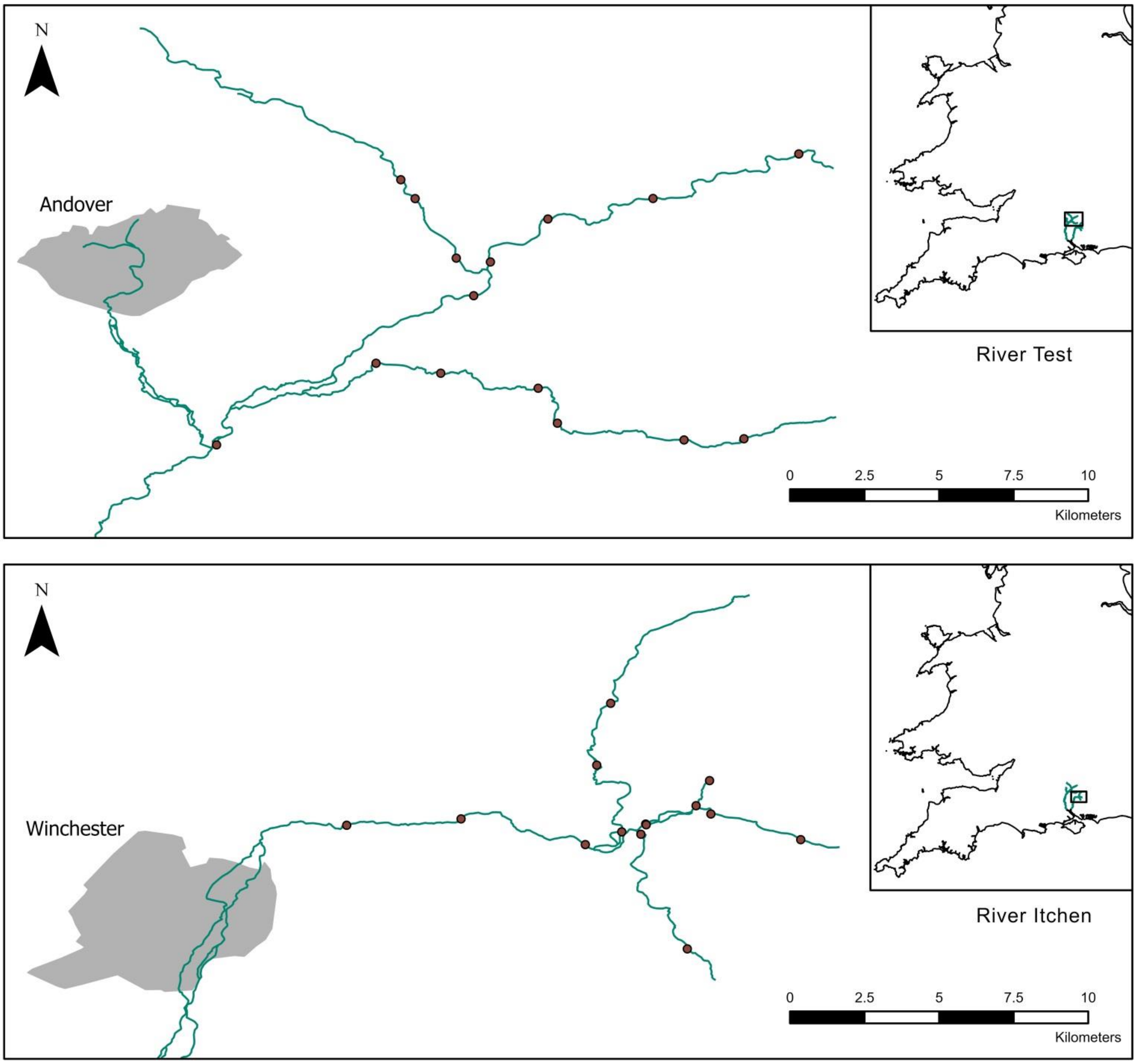

Fig. 1. The locations of the upper catchments of the Rivers Itchen and Test (insets) and locations of sampling sites used in this study (red symbols).

2.2 Sampling and sample storage

Water samples were collected from 14 locations in each catchment, including tributaries and main channels (Fig. 1). Samples were collected twice during periods of relatively low flow (04/07/2013 & 24/08/2013) and twice during periods of relatively high flow (27/11/2012 & 17/01/2013). Surface (<20 cm) samples were collected from mid-channel or at least 1 m from the river bank to avoid marginal waters. Samples were transferred *in situ* to high-density PTFE containers (USEPA, 2012a) pre-rinsed with dilute acid (HCl or H_2SO_4) to reduce adsorption to internal surfaces (Jarvie *et al.*, 2002; Nollet & De Gelder, 2013; USEPA, 2012b). Samples were stored in darkness at 4^{o}C before analysis; filtration was carried out within 12 hours of sampling and analysis within 48 hours of sampling to avoid changes in the sample composition (Fishman *et al.*, 1986; Kotlash & Chessman, 1998).

2.3 Sample analysis

Concentrations of P were determined in three size ranges. Samples were size-fractionated by filtration and analysed colourimetrically, with or without oxidative digestion, to determine unreactive and reactive P.

2.3.1 Determination of reactive and unreactive P

The phospho-molybdenum blue method (Murphy & Riley, 1962) was used to determine P concentrations. Phosphate reacts with molybdate ions under acidic conditions to form 1,2-molybdophosphoric acid that is reduced by ascorbic acid to form the phospho-molybdenum blue complex which absorbs light strongly at 880 nm. Detection limits for this method are typically of 5–10 μgPL^{-1} *(*Jarvie *et al.*, 2002). This study used a segmented flow analyser (Bran+Luebbe Auto Analyzer 3); the limit of detection (Armbruster & Pry, 2008) was 4 μgPL^{-1}.

P that reacts with molybdate under analytical conditions is customarily termed “reactive” P *(*Jarvie *et al.*, 2002). To determine all P, including forms that do not react readily with molybdate under analytical conditions, oxidative digestion was applied (Maher and Woo, 1998); a high temperature, high acidity oxidising environment releases and converts phosphorus to orthophosphate. This study employed acid-persulphate digestion using autoclave heating (>120°C for >30 minutes) for batch analysis (Jarvie *et al.*, 2002).

2.3.2 Size fractionation of P

In principle, size fractionation differentiates soluble and particulate forms of P (House, 2003; May *et al.*, 2001; Neal *et al.*, 2000); 0.45 μm filters are often used for this purpose. Operational distinctions between size fractions are not necessarily clear or unambiguous (Haygarth *et al.*, 1997; Hens and Merckx, 2002), but recognise the principle that a continuum of materials can be separated on the basis of size (Gregory, 2006). The present study examined three size fractions, differentiated by filtration: “soluble” <0.22, “intermediate” 0.22 – 0.7 μm, and “particulate” >0.7 μm.

2.3.3 Fractions of P determined and derived

Samples were left unfiltered, or filtered (0.22 µm or 0.7 µm) to determine of P of different size classes (§2.3.2), and analysed colourimetrically with or without oxidative digestion (§2.3.1). Concentrations of reactive and unreactive P within of soluble (<0.22 µm), intermediate (0.22-0.7 µm) and particulate (>0.7 µm) size were derived by difference (Table 1).

Filtration	Oxidative Digestion	P components determined
0.22 µm filtrate	Undigested	SRP
	Digested	SRP + SUP
0.7 µm filtrate	Undigested	SRP + IRP
	Digested	SRP + IRP + SUP + IUP
Unfiltered	Undigested	PRP + IRP + SRP
	Digested	PRP + IRP + SRP + PUP + IUP + SUP

Table 1 P fractions determined in relation to the procedures applied. Fractions relate to size (Soluble: S; Intermediate: I; Particulate: P; Total: T; §2.3.2) and reactivity (Reactive: R; Unreactive: U; §2.3.1).

2.4 Quantitative analyses

2.4.1 Statistical analyses

Tests were applied to establish the significance of differences using Kruskal-Wallis One Way Analysis of Variance combined with Pairwise Multiple Comparison Procedures (Tukey Test) and using SigmaPlot v13 (Systat Software Inc.). In all cases data were non-parametric (Shapiro-Wilk Normality Test).

2.4.2 Classification of water quality in relation to P fractionation

Data on P fractions were analysed to determine how the selection of P fractions influences the classification of water quality. The Water Framework Directive (WFD; 2000/60/EC; EU, 2000) targets for SRP in the upper Itchen were applied as illustrative benchmarks. Five WFD (2000/60/EC; EU, 2000) targets for the River Itchen are prescribed for individual water bodies; these fall within a discrete range. The thresholds for the upper Itchen range from 82 to 88 µgPL^{-1} for "good" WFD status, and 44 to 48 µgPL^{-1} for "high" WFD status (Morse, 2017). As indicative threshold values, concentrations of 45 µgPL^{-1} and 85 µgPL^{-1} were selected. To determine how inclusion of intermediate and particulate P alters the water quality classification, the proportions of samples with SRP within threshold ranges (<45 µgPL^{-1} and 45 to 85 µgPL^{-1}) were first calculated. This process was repeated (1) for SRP and IRP (RP<0.7 µm), (2) for all RP (SRP+IRP+PRP), and (3) total P (RP and UP in all size fractions) to determine to influence of sequentially extending the suite of P fractions included.

3. Results

3.1 P fractions in the upper Itchen and Test catchments

Ternary plots were employed to visualise the composition of P across size fractions and to compare reactive and unreactive P. For the Itchen, RP (Fig. 2A) was dominated by SRP, which accounted for more than 80% of the TRP in 71% of the samples. TRP ranged from 6 to 175 µgPL^{-1}, with median, 25th percentile and 75th percentile concentrations of 51, 35 and 75 µgPL^{-1} respectively. One outlier was observed, in which IRP accounted for 91% of the TRP (Fig. 2A).

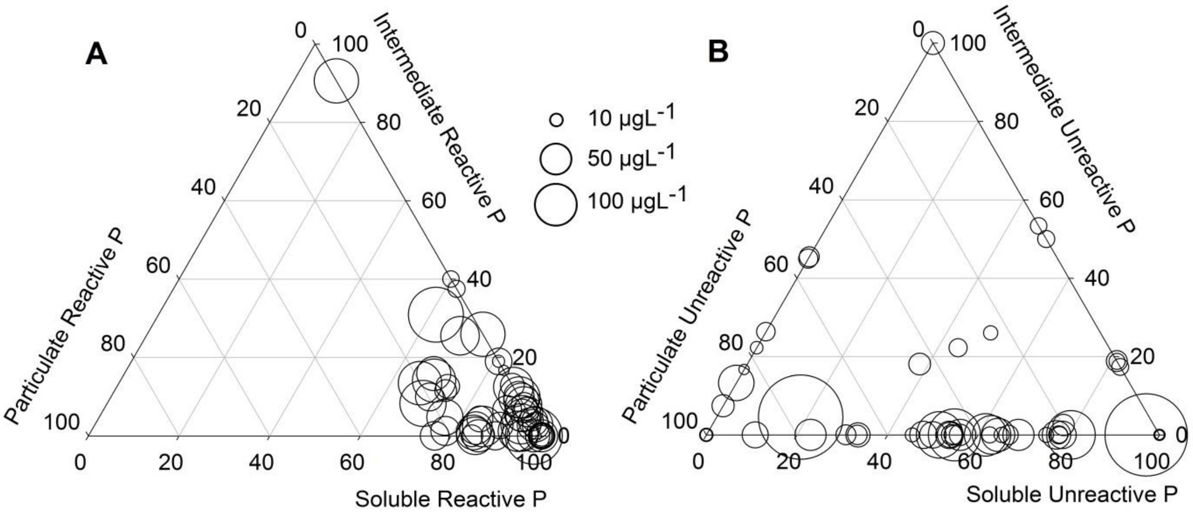

Fig. 2. The composition of **A** reactive and **B** unreactive P for samples from the upper Itchen (2013-2014). The position of each symbol indicates the relative proportions (%) of soluble, intermediate and particulate P; the area of each symbol is proportional to the concentration of total reactive P (**A**) and total unreactive P (**B**).

Unreactive P in the Itchen samples was dominated by SUP and/or PUP (Fig. 2B). TUP ranged from 5 to 383 µgPL^{-1}, with median, 25th percentile and 75th percentile concentrations of 22, 13 and 41 µgPL^{-1} respectively. Contributions of IUP to TUP were generally lower than for SUP or PUP, and were highly variable (Fig. 2B). As for RP in the Itchen samples (Fig. 2A), one outlier was observed for which IUP accounted for 100% of TUP (Fig. 2B).

RP in samples from the Test (Fig. 3A) was also dominated by SRP, which accounted for over 80% of the TRP in 75% of the samples. TRP ranged from 15 to 194 µgPL^{-1}, with median, 25th and 75th percentile concentrations of 41, 28 and 51 µgPL^{-1} respectively. Two outliers were observed, one in which IRP accounted for 90% of the TRP (Fig. 3A) and another in which PRP accounted for 62% of TRP and SRP for 24% (Fig. 3A).

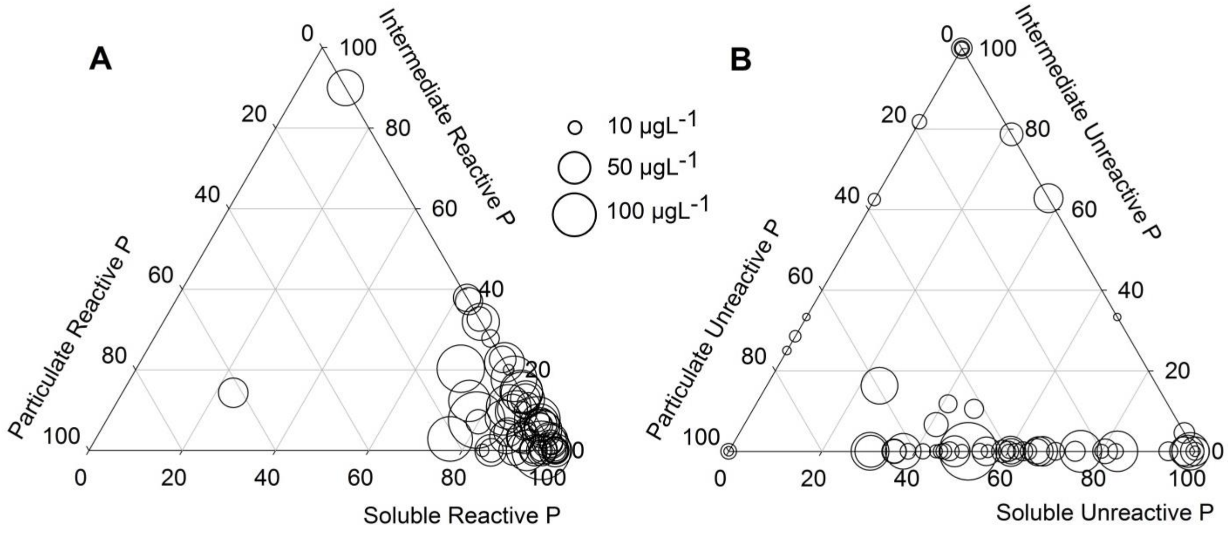

Fig. 3. The composition of **A** reactive and **B** unreactive P for samples from the upper Test (2013-2014). The position of each symbol indicates the relative proportions (%) of soluble, intermediate and particulate P; the area of each symbol is proportional to the concentration of total reactive P (**A**) and total unreactive P (**B**).

UP for the Test was also similar to the Itchen. SUP and/or PUP dominated UP in most samples (Fig. 3B). For samples not dominated by SUP and PUP, UP tended to comprise either PUP and IUP or SRP and IUP (Fig. 3A). TUP ranged from 3 to 168 μgPL^{-1}, with median, 25th percentile and 75th percentile concentrations of 41, 28 and 51 μgPL^{-1} respectively. Contributions of IUP to TUP were highly variable and generally lower than for SUP and PUP (Fig. 3B). In several samples, UP was entirely within the soluble fraction (Fig. 3B).

Notwithstanding outliers (Figs. 2 & 3), there were commonalities for the Itchen and Test. SRP tended to dominate RP, and contributions of IRP and PRP were generally low (Figs. 2A & 3A). For UP, SUP and PUP tended to contribute more to TUP than IUP; contributions of IUP to TUP were generally lower, if somewhat variable (Figs. 2B & 3B).

3.2 P fractions in relation to water quality classification

Data were analysed to determine how choices of P fraction(s) for monitoring purposes influence the outcomes of water quality monitoring (§2.4.2). Comparisons were made between SRP, SRP+IRP, TRP (SRP+IRP+PRP) and TP (Table 1).

Statistical analysis (Kruskal-Wallis one-way ANOVA on ranks; Tukey test pairwise multiple comparison) demonstrated that (1) there were no statistically significant differences between SRP, SRP+IRP and TRP (SRP+IRP+PRP) for either the Test or the Itchen, and (2) TP were significantly higher than all other measures of reactive P (Fig. 4).

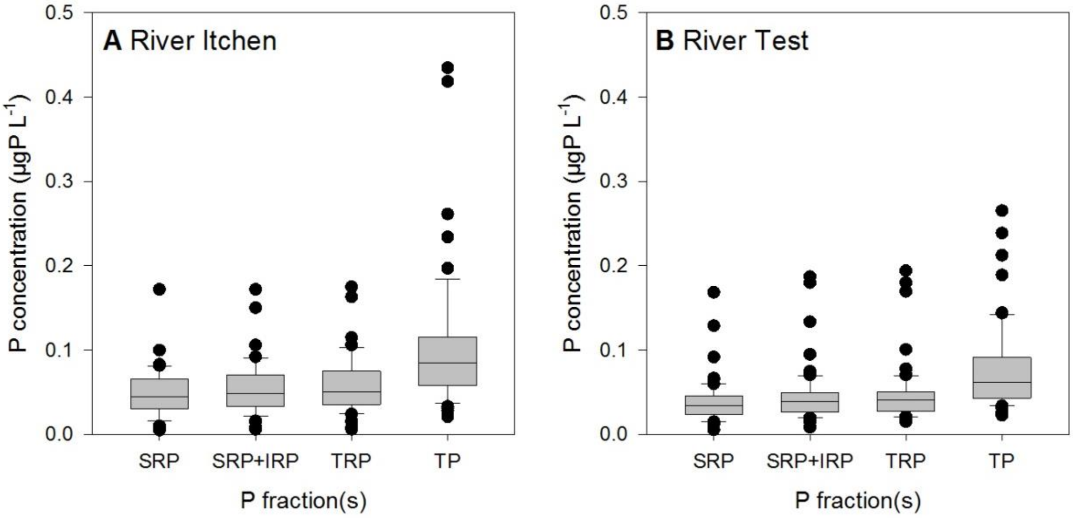

Fig. 4. Distributions of P fractions and their combinations for **A** the Itchen and **B** the Test. Boxes show medians, 25th and 75th percentiles; whiskers show 5th and 95th percentiles. Outliers are also indicated (●). SRP: Soluble RP (SRP); IRP: Intermediate RP; TRP: Total RP (SRP+IRP+PRP); TP: total P (all forms).

Considered in relation to indicative Water Framework Directive (EU, 2000) targets (§2.4.2), the P data for the Itchen and Test demonstrated that inclusion of intermediate and particulate RP, in addition to SRP, alters the resultant profile of water quality status (Table 2). For SRP alone, 75% of samples from the Test and 50% from the Itchen were <45 µgPL^{-1} (Table 2). When intermediate and particulate RP were also included, the proportions of samples with RP of <45 µgPL^{-1} were reduced from 50% to 41% for the Itchen, and from 75% to 62% for the Test (Table 2). For the Itchen, sequential inclusion of IRP and PRP led to fewer samples with P in both the <45 µgPL^{-1} and the 45 - 85 µgPL^{-1} ranges, and to more samples with RP > 85 µgPL^{-1} (Table 2). The sequential inclusion of IRP and PRP for the Test samples led to fewer samples with P <45 µgPL^{-1}, more samples in the 45 - 85 µgPL^{-1} range, and little change in numbers of samples > 85 µgPL^{-1} (Table 2).

Water Framework Directive quality class	**P threshold or range (µgL^{-1})**	**P fractions included**			
		SRP	**SRP+IRP**	**PRP**	**TP**
River Itchen					
“High”	<45	50%	45%	41%	18%
“Good”	45 – 85	46%	44%	39%	32%
Less than “good”	>85	4%	11%	20%	50%
River Test					
“High”	<45	75%	68%	62%	32%
“Good”	45 – 85	20%	25%	31%	40%
Less than “good”	>85	5%	7%	7%	28%

Table 2 Proportions (%) of surface water samples with P concentrations corresponding to Water Framework Directive (EU, 2000) water quality class of “good” or “high” standard. The P threshold and range are indicative values based on those that apply to waterbodies within the upper Itchen catchment (Morse, 2017). SRP: RP <0.22 µm; SRP+IRP: RP <0.7 µm; PRP: all reactive P in all size fractions; TP: total P (reactive plus unreactive P in all size fractions).

4. Discussion

4.1 P fractions in chalk streams

This study aimed first to elucidate the contributions of different size fractions of RP and UP to P in chalk stream surface waters. In the upper Itchen and Test, SRP dominated RP (Figs. 2A & 3A): for both rivers, over 80% of the RP was <0.22 µm in over 70% of the samples. P fractions >0.22 µm contributed more than a fifth of the RP pool in *ca.* 25-30% of samples (Figs. 2A & 3A). Domination of RP by intermediate or particulate size fractions was observed, albeit rarely, in samples from both rivers (Figs. 2A & 3A).

Compared with other English chalk streams, the upper Test and Itchen appear unexceptional in terms of surface water P (Table 3). Where fractionated by filtration, commonly at 0.45 µm (Demars & Harper, 2005; Evans & Johnes, 2004), P concentrations in other English chalk streams are highly variable: maxima and minima differ considerably (Table 3). SRP tends to dominate the soluble P pool (Table 3), as has been observed in other UK rivers (91%; Jarvie *et al.*, 1998) as well as chalk streams (81%; Halliday *et al.*, 2014). SRP and SUP were of similar magnitude in the River Wensum, a chalk stream, where only diffuse sources of P were present (Demars & Harper, 2005).

Studies reporting both SRP and TRP for chalk streams appear infrequent (Table 3). When recorded (e.g. Halliday *et al.*, 2014), TRP is dominated by SRP (Table 3): 84-87% of TRP typically comprises SRP (Table 3). A linear correlation between SRP and PRP for the River Enbourne inferred a SRP:PRP ratio of 0.8:1 (Halliday *et al.*, 2014). Removal of particulate material from a chalk stream water sample will thus likely exclude *ca.* 15-20% of the TRP. This ratio is not consistent: for the Itchen and Test, SRP occasionally accounted for <10% of the TRP (Figs. 2a & 3a; Table 3).

The proportion of TP attributable to SRP was highly variable (Table 3). For the Test and Itchen, SRP typically accounted for *ca.* 55% of TP and ranged from <10% to >85% (Table 3). For other chalk streams, SRP accounts for 15% to 78% of TP (Table 3). For sites the on the River Wensum impacted by effluents from a nearby large STW, SRP was high relative to both TP (*ca.* 70%; Table 3) and SUP (Demars & Harper, 2005), indicating an association between P fractionation and P sources (Edwards & Withers, 2007). Such associations demonstrate the value of analysing a range of P fractions as a means to identify sources of P to rivers and thereby inform management decisions.

For the Test, Itchen and other English chalk rivers, size fractionation of RP and UP (Figs. 2 & 3; Table 3) demonstrates that contributions of P fractions are highly variable. TP comprises an often inconsistent mix of UP and RP of different size; filter–retained RP fractions augment SRP. The implications of fractionation are thus (1) consideration of SRP as the sole measure of reactive P omits other forms of RP, and (2) the lack of a consistent relationship between SRP and other P measures undermines the use of SRP as a proxy for overall water column P.

River	Value	SRP	SUP	PP	TRP	TP	SRP/TRP (%)	SRP/TP (%)
Itchen[1] (Hampshire, UK)	Mean	48	23	22	58	103	84	54
	Range	5-172	0-353	0-294	6-175	21-435	9-100	4-87
Test[1] (Hampshire, UK)	Mean	39	17	12	47	77	86	55
	Range	5-168	0-86	0-82	15-194	22-265	10-100	10-92
Wensum[2] (Norfolk, UK)	Mean[3]	9	10	30	ND	48	-	19
		14	10	15		39		36
		15	13	20		47		32
		14	12	50		77		18
	Mean[4]	49	12	18	ND	79	-	62
		55	11	31		97		57
	Mean[5]	141	12	56	ND	210	-	67
		282	19	150		451		63
		384	15	95		493		78
Lamborne[6] (Berkshire, UK)	Mean	87	62	33	ND	182	-	47
		71	51	21		143		50
	Range	42-179	0-124	0-278	ND	-	-	-
Ebble[7] (Wiltshire, UK)	Mean	80	50	30	ND	160	-	50[8]
		50	50	70		170		29[8]
		90	70	470		610		15[8]
		80	50	270		410		20[8]
Wylye[7] (Wiltshire, UK)	Mean	140	50	40	ND	230	-	61[8]
		90	60	90		240		38[8]
		90	50	40		180		50[8]
		100	50	40		180		56[8]
Enbourne[9] (Berkshire, UK)	Mean	130	30[10]	40	150	200	87[8]	65[8]
	Range	20-31	ND	20-120	0-600	90-450	-	-

Table 3 Water column P data for selected chalk streams in England determined using the molybdate method. Unless otherwise stated, soluble and particulate fractions were differentiated by use of 0.45μm filter. [1] The present study: SRP and SUP in 0.22 μm filtrates; 14 sites sampled four times over 2 years. [2] Demars & Harper (2005): 9 sites sampled October 2000, January, April and June 2001; acid persulphate digestion; means for all samples; TP and SRP/TP data based on mean SRP and TP (Table 2); [3] four sites impacted only by diffuse sources; [4] two sites impacted by small, distant point source effluents; [5] three sites impacted by large nearby sewage treatment works effluents. [6] Evans & Johnes (2004): daily samples from 2 sites, March 1998 to March 2000; P microwave persulphate oxidation and auto-analyser; mean values for the sampling period; TP and SRP/TP data are based on mean data for SRP, SUP and PP (Table 2). [7] Lloyd *et al.* (2019): daily samples from two sites in each river, January 2012 to March 2014; persulphate oxidation and an auto-analyser; annual means (2012 and 2013) for each monitored site; [8] estimated from annual means. [9] Halliday *et al.* (2014): hourly and weekly data for one site, November 2009 to February 2012; [10] estimated as the difference between mean SRP and mean total dissolved P (TDP). ND: not determined. All data are in μgPL^{-1}.

4.2 P fractions in relation to regulation and water quality classification

Regulatory frameworks (e.g. JNCC, 2016; UKTAG, 2013) often focus upon a subset of P fractions, typically comprising SRP in filtered samples or RP in samples after settlement of suspended solids. The present study evaluated the impacts of extending the range of P fractions on the appraisal of water quality status (Fig. 4; Table 2); two outcomes were observed. First, on a statistical basis, there was no difference between SRP, SRP+IRP and TRP; a significant difference was only observed when unreactive forms of P were also included (Fig. 4). Secondly, including a wider range of RP size fractions (Fig. 4) led to concentrations that were sufficiently higher to change the water quality classification (Table 2), notably when concentrations of SRP were close to threshold values. The nett impact of this change was a general downturn in water quality class (Table 2).

Changing the suite of RP fractions included in water quality class assessment alters the assessment criteria and different outcomes may result. Boundaries for water quality class have been altered previously: lowering thresholds for annual mean RP for "high", "moderate" and "good" class (EU, 2000) led to the number of "high" class sites across England falling from 54% to 38% (UKTAG, 2013). In the present study, the number of samples of "high" water quality decreased when RP included IRP and PRP in addition to SRP: 9% and 13% fewer samples were consistent with high quality for the Itchen and Test, respectively (Table 2).

4.3 Implications for the regulatory management of chalk streams

4.3.1 P analysis in the context of regulatory management

This study demonstrates the quantitative importance of a wide range of P fractions in chalk stream surface waters, and the sensitivity of water quality assessment to the inclusion of filter-retained P fractions. Determination of P in the UK regulatory context relies on operationally-defined measures of P. In water samples from which particulate materials have been allowed to settle, RP in the supernatant comprises molybdate-reactive P (MRP) remaining in suspension. Filtration also removes particulate materials. Settlement and filtration have a common purpose, i.e. the removal of particle-associated RP, but both measure "fractions" of P (Jarvie *et al.*, 2002) and outcomes are without exact meaning in terms of chemical speciation or form. Particulates are usually allowed to settle over a period of "a few hours" (EA, pers. comm.): which materials are removed from the supernatant over this time depends on the settling velocity. Ulén (2004), for example, reported settling velocities of 0.68 to 0.0034 cm day^{-1} for P-containing particles from agricultural drains; colloids and clays in particular had slow settling rates and would likely remain in the supernatant unless allowed to settle for several days. Filtrates may also contain small particles with which P is associated. In lake waters on limestone geology (chemically similar to chalk), orthophosphate associated with particulates of up to 0.4 μm in the presence of iron (Shaw, 1994) that would pass through a 0.45 μm filter but are clearly not of dissolved form.

There remains also uncertainty regarding the meaning of SRP. Reagents used in colourimetric analysis may lead to over-estimates of inorganic P (Ballantine *et al.*, 2009). More meaningful assessment of the potential for biological assimilation of P may be gleaned by extraction procedures (Dorich *et al.*, 1985) or bioassays (Edwards & Withers, 2007) that apply processes specifically intended to determine directly the bioavailability of P.

The use of RP or SRP as a means to monitor water quality does, however, benefit from broad-scale and long-term application that provides continuity and comparability. Underpinning evidence (Mainstone, 2010) is consistent with the standards as set (JNCC, 2016; UKTAG, 2013, 2014) in that both employ common measures of P. If RP in filtered or settled samples does not represent the true concentration of bioavailable P and provides an imperfect measure on an analytical basis, uncertainties in this regards should in principle be systematic.

The meaning and interpretation of RP in biological terms has been questioned, but there are well-established empirical and causal links between RP and its ecological impacts (Jarvie *et al.*, 2006; Mainstone, 2010). The relationship between biological response and P is highly complex, being affected by many factors (Hilton *et al.*, 2006; O'Hare *et al.*, 2019; UKTAG, 2013). There are consequently variations in and uncertainties regarding biological responses to P: thresholds applied in water quality classification recognise the presence and influence of scatter within underpinning data (UKTAG, 2013).

How RP is fractionated is also critical. RP occurs across a spectrum of sizes (Figs. 2 & 3; Table 3), but the meaning of separation by filtration of soluble and particulate material is equivocal (Haygarth *et al.*, 1997; Hens and Merckx, 2002) and to an extent arbitrary (Gregory, 2006). P in freshwaters is associated with materials across a continuous size spectrum (Jones *et al.*, 1993; Shaw *et al.*, 1992, 1996, 2000; Hens & Merckx, 2002) rather than within discrete and distinct size classes.

4.3.2 Recommendations for the regulatory management of P

We propose that measurements of P and related thresholds for water quality classification suitable for regulatory purposes should be:

(1) *Meaningful* Measure(s) of P should be related or relatable to effects of P *in situ*, i.e. indicative of the availability of P to lotic photosynthetic organisms (Boström *et al.*, 1988; Mainstone & Parr, 2002; Whitton & Neale, 2011).

(2) *Deliverable* Resource constraints infer that rapid, simple and inexpensive measurements of P are desirable (Jarvie *et al.*, 2013) (§1.3.2).

(3) *Evidence-based* Thresholds for water quality class should be based upon evidence linking chemical variables for water quality to the ecological status of rivers (Mainstone, 2010).

Established methods of determining RP or SRP provide information on procedurally-defined fractions of P comprising a mixture chemical species (Jarvie & Neale, 2002); the meaning of RP or SRP is inexact and determining bioavailable P on the basis of chemical analysis remains challenging (Boström *et al.*, 1988). The removal of particulate materials adds further complications. P associated with particles may be released into the water column (Ballantine *et al.*, 2009; Evans & Johnes, 2004; Evans *et al.*, 2004); the removal of particles can eliminate potentially bioavailable RP. For the Test and Itchen, removal of particles >0.22μm typically reduced the RP pool by 15-20% (Figs. 2A & 3A). There are benefits to discriminating between filterable and particulate forms of P. The potential fate of P is influenced by the likelihood of settlement: particulate fractions of P have the potential to influence in-river storage of P (Ballantine *et al.*, 2009; Evans & Johnes, 2004; House, 2003; Wang and Li, 2010) and downstream transport (Demars *et al.*, 2005). Secondly, information regarding P within soluble and particulate fractions can provide insight to P sources (e.g. Demars & Harper, 2005; Edwards & Withers, 2007).

Methods of separating particulate P fractions have contrasting merits. Filtration is inexpensive and demands relatively little time; particle removal should be consistent and repeatable. Settlement of particles is influenced by the settling velocity and the time permitted for settling. Outcomes of particle settlement are, arguably, inconsistent as the sample-specific composition and characteristics of particles potentially influence settlement velocity (Ulén, 2004). In practice, the time allowed for settlement of particles is not prescribed (EA, Pers. comm.); standardisation is desirable. Discrimination between fractions of P likely to remain in or settle out of the water column can provide insight to their potential fate. The use of laboratory-based settlement has merit in this regard, indicating potential loss of P from the water column.

Notwithstanding concerns regarding the meaning of P fractions determined for regulatory purposes (§1.3.2), there is long-term and extensive empirical evidence linking RP to ecological impacts (Jarvie *et al.*, 2006; Mainstone, 2010). Biological responses to P can be uncertain and variable; scatter in the data are taken into account in setting thresholds for water quality classification (UKTAG, 2013).

The approach to determination of water quality with regard to P employs a system that is imperfect but has merit. Measuring P in a manner that indicates its bioavailability is a challenge that is not restricted to the regulatory context; empirical evidence of the ecological impacts of P is of particular value. If the established approach were to be replaced, an alternative or replacement evidence base would be needed; without access to long-term and extensive data the value of a revised approach to regulating P would likely lack robustness.

We propose a pragmatic approach for routine monitoring based on the determination of RP after first allowing suspended solids to settle (UKTAG, 2013). This approach repeats the recommendation of the UKTAG (2013), but we propose two adjustments: (1) settlement of samples uses a standardised period to allow settlement of particles in the sample, and (2) a pipette or syringe is used to remove aliquots of supernatants for colourimetric analysis to avoid disturbance of settled particles. Maintaining the use of RP as a default measure of P in surface waters, notwithstanding its limitations, aligns with established practice and thus builds upon the substantive research and analysis (e.g. Mainstone, 2010) that underpins regulatory frameworks (JNCC, 2016; UKTAG 2102, 2013, 2014), and provides continuity and comparability in monitoring and regulation.

Settling of samples rather than filtration is proposed:
(1) Neither settlement nor filtration guarantees isolation of specific forms of phosphorus. RP in filtrates and supernatants comprises readily hydrolysable P that cannot be assumed to be exclusively P in the form of orthophosphate and lacks specificity.
(2) Partitioning of P between settled solids and forms remaining in suspension provides insight to the potential for settlement of suspended materials in areas of sluggish flow and their subsequent influence on surface water P (Ballantine *et al.*, 2009; House, 2003; Mainstone *et al.*, 2000).

This approach ensures an ongoing framework that delivers broad-scale evaluation of the status of chalk streams. Information thus acquired also has value in terms of management; the association between P sources and observed P fractions (Edwards & Withers, 2007) can provide insight to guide and inform remediation. High levels of SRP that dominate the TP pool, for example, can indicate the influence of STWs upon surface water P (e.g. Demars & Harper, 2005). Such data can be readily provided by extant regulatory monitoring.

In other instances, insight gained via routine monitoring may be lacking. In such cases we propose additional "diagnostic" monitoring in which sampling and analysis are extended, with the intention of informing management measures. Given the resource implications of extended sampling and analyses, diagnostic monitoring is only proposed when circumstances merit. Specifically, it is recommended that diagnostic monitoring be reserved for when anomalies are identified that cannot be readily explained on the basis of data from routine monitoring. Regulatory management of riverine P assumes that levels of P and the overall status of a river are commensurate (Mainstone, 2010; UKTAG, 2013). If circumstances arise in which this fundamental relationship is decoupled, diagnostic monitoring is merited, e.g.: (1) if the ecological condition of a river is inconsistent with that expected on the basis of observed RP concentrations (UKTAG, 2013); (2) if marked reductions in river water RP do not lead to ecological improvements (e.g. Jarvie *et al.*, 2013), and (3) if mitigation measures result in slower or lower magnitude improvements in water quality than expected (e.g. Sharpley *et al.*, 2013).

When circumstances merit diagnostic monitoring, we advocate a stepwise approach. First, an inventory should be made of potential sources of P within the catchment (e.g. Morse, 2017), including agriculture (arable, livestock, watercress and fish), sewage systems (sewered, unsewered, STWs and discharges), land use, and other activities. Records of water quality and composition should also be collated at this stage. This inventory should then be augmented by a desk-based study to identify, where possible, the types of P that might be expected to be released from the catchment sources present and their temporal mode of release.

A bespoke programme of sampling and analysis should be based on the inventory and desk-based study, addressing (1) forms of P that are of concern, and (2) needs for sampling. Catchment sources that release P in forms that are not routinely monitored (§1.3.3) should be prioritised, information regarding other P fractions being already available. Regarding temporal aspects, the inventory and desk-based study should evaluate potential sources of P in term of their potential for chronic or acute impacts of P on surface waters. In the case of acute discharges, monthly sampling (UKTAG 2013, 2014) is unlikely to indicate the presence, duration and severity of sporadic events; site-specific and high frequency sampling may be necessary (Fones *et al.* 2020). Consideration should also be given to seasonally-dependent facets; periods of higher insolation can influence the uptake of P by photosynthetic organisms, which in turn affects the balance between P fractions of different size and reactivity.

5. Conclusions

This study demonstrates the complexity and variability of P fractionation in chalk streams and reinforces the challenges of implementing regulatory management whilst balancing the needs for scientific meaning and resource constraints. It is recommended that monitoring comprises complementary elements: routine monitoring and diagnostic monitoring. We propose that analytical processes for monitoring harmonised, with particulate materials being allowed to settle out of suspension prior to colourimetric analysis for a prescribed period. Where there is an apparent decoupling between the levels of reactive P present and the status of a river and/or mitigation measures undertaken do not result in predicted outcomes, we recommend implementation of diagnostic monitoring. A catchment inventory and desk-based research lead to a programme of sampling and analysis that is targeted to potential sources of P identified, and with the intention of providing evidence to guide remediation.

6. References

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