UNIVERSITY OF SOUTHAMPTON

FACULTY OF NATURAL AND ENVIRONMENTAL SCIENCES

School of Ocean and Earth Sciences

Volume 1 of 1

INVESTIGATION INTO THE DYNAMICS OF DISSOLVED ORGANIC PHOSPHORUS CONCENTRATIONS OVER AN ANNUAL PERIOD IN A RIVER AND ESTUARY SYSTEM

by

Jack Billinge

Thesis for the degree of Doctor of Philosophy

September 2016

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF NATURAL AND ENVIRONMENTAL SCIENCES

Ocean and Earth Sciences

Thesis for the degree of Doctor of Philosophy

INVESTIGATION INTO THE DYNAMICS OF DISSOLVED ORGANIC PHOSPHORUS CONCENTRATIONS OVER AN ANNUAL PERIOD IN A RIVER AND ESTUARY SYSTEM

Jack Billinge

Dissolved organic phosphorus (DOP) may play an important role in the control of primary production in aquatic and coastal systems. However, whereas dissolved inorganic phosphorus is routinely measured in most UK rivers and estuaries by the Environment Agency, DOP is not determined and there are few high frequency temporal records of how DOP concentrations vary seasonally and in relation to changes in river flow. The aim of research presented in this thesis was to investigate the DOP dynamics in the lower reaches of two major UK south coast rivers (the Hampshire Avon and Dorset Stour) and the receiving waters of the Christchurch Harbour estuary over an annual period at a high (i.e. weekly) sampling frequency. Water samples were collected from 1 site on the Hampshire Avon river (Knapp Mill), two sites on Dorset Stour river (Throop and Iford Bridge), and one site at Christchurch Harbour estuary (Mudeford Quay) on a weekly basis from April 2013 to April 2014.

In order to investigate the concentrations and fluxes of DOP in the two rivers and the estuary, a new method of analysis of total dissolved phosphorus (TDP) using inductively coupled mass spectrometry (ICP-MS) was implemented, in addition to a well described spectrophotometric technique to measure soluble reactive phosphorus (SRP). The DOP concentration was then assumed to be the difference between the TDP concentration and the SRP concentration. ICP-MS analysis of certified reference materials containing known concentrations of TDP indicated that the technique was detecting TDP to within 5% of the expected concentration.

DOP concentrations were highest in the River Stour, with the highest concentrations detected at Throop (up to 12.1µM) and Iford Bridge (up to 21.0µM) on the River Stour. The River Avon had the lowest DOP concentration of the four sites (up to 1.45µM). The estuarine site at Mudeford Quay was high in DOP concentration in the summer (ranging between 5.7µM and 18.8µM), with lower concentrations in the winter (ranging from undetectable concentrations to

2.8µM). The DOP concentration at all of the sites decreased during periods of high river flow, most likely due to dilution of DOP by rainfall.

During periods of high river flow increased fluxes of TDP, SRP and DOP occurred in both rivers flowing into the Christchurch Harbour estuary, with 45.2% of the annual SRP flux at Knapp Mill and 49.5% at Throop occurring during an extended period of high river flow from 16/12/13 to 27/3/14. Riverine fluxes of DOP at Knapp Mill and Throop were highest over periods of high river flow during January and February 2014 when river flows were sustained at peak values over 69m^3/s and 50m^3/s respectively for several weeks. At Knapp Mill, 50% of the annual flux of DOP occurred over a 9-week period of high river flow in early 2014.

During summer 2014 several transect surveys of Christchurch Harbour were conducted to investigate the relationship between TDP, SRP and DOP with salinity. On most dates TDP and SRP showed non-conservative removal of phosphate between the riverine and seawater end member concentrations. DOP concentrations on some dates showed a non-conservative removal of phosphate at low salinities whereas on other dates DOP showed a small increasing linear relationship with salinity.

In addition to TDP, SRP and derived DOP concentrations, weekly measurements of nitrate and silicate concentrations were analysed on samples collected from each site as well as measurements of water quality parameters including temperature, conductivity/salinity, chlorophyll a concentration and turbidity. Mean dissolved inorganic nitrate concentrations over the sampling period were 391.6µM at Knapp Mill, 508.9µM at Throop, 545.6µM at Iford Bridge and 319.3µM at Mudeford Quay. Mean silicate concentrations over the sampling period were 135.4µM at Knapp Mill, 104.3µM at Throop, 112.8µM at Iford Bridge and 84.1µM at Mudeford Quay. Highest concentrations of inorganic nutrients were seen over summer, with the lowest seen in winter.

The results from this study have provided new data on the seasonal changes in DOP in comparison to SRP measured at weekly intervals in two contrasting south coast UK rivers that feed into the small shallow eutrophic estuary of Christchurch Harbour. These high frequency measurements have provided new insight into the processes influencing the flux of dissolved phosphate from temperate rivers into estuaries highlighting the importance of including analysis of organic forms of nutrients when assessing the nutrient status of natural waters.

Contents

List of figures vii

List of tables xi

Declaration of authorship xiii

Acknowledgements xiv

Definitions and Abbreviation xv

Chapter 1: Introduction 1

- **1.1 Phosphorus** 2
- **1.2 Nitrogen** 8
- **1.3 Silicon** 10
- **1.4 Estuaries** 11
- **1.5 Research area** 16
- **1.6 NERC Macronutrients consortium** 19
- **1.7 Thesis overview** 20
  - 1.7.1 Research questions 20
  - 1.7.2 Aims and Objectives of the thesis 21

Chapter 2: Inorganic Nutrients in the Christchurch Harbour System 24

- **2.1 Introduction** 24
- **2.2 Methods** 26
  - 2.2.1 Sample collection 26
  - 2.2.2 Filtration 27
  - 2.2.3 Analysis 28
- **2.3 Results** 29
  - 2.3.1 Temperature 29
  - 2.3.2 Conductivity/Salinity 29
  - 2.3.3 Dissolved Oxygen Saturation 30
  - 2.3.4 Chlorophyll a concentration 30
  - 2.3.5 pH 31
  - 2.3.6 Turbidity 31
  - 2.3.7 Mean daily flow rate 36
  - 2.3.8 Nitrate concentration 38

2.3.9 Phosphate concentration 42

2.3.10 Silica concentration 44

2.3.11 Nutrient fluxes 46

2.3.12 Historic Nutrient data 51

2.4 Discussion 55

2.4.1 Physiochemical parameters 55

2.4.2 Nitrate 56

2.4.3 Phosphate 58

2.4.4 Silica 60

2.4.5 Historic nutrient data 61

2.4.6 Fluxes of nutrients 62

2.4.7 Conclusions 63

Chapter 3: Optimization of the determination of dissolved organic phosphorus in environmental water samples using a combination of ICP-MS and spectrophotometric colourimetric analysis. 66

3.1 Introduction 66

3.2 Methods 69

3.2.1 Sample collection 69

3.2.2 Filtration and storage 69

3.3 ICP-MS Methods and method development 70

3.3.1 Instrumental 70

3.3.2 Standard addition 74

3.3.3 Analysis of organic phosphorus standards using ICP-MS 77

3.3.4 Certified reference material for ICP-MS analysis 79

3.3.5 Effects of freezing the sample 81

3.3.6 Effects of filtration on sample 84

3.3.7 Effects of chemical oxidation on water sample prior to ICP-MS analysis 86

3.4 SRP Methods 88

3.4.1 Automated Inorganic phosphate analysis 89

3.4.2 Repeatability of the SRP analysis 90

3.4.4 Chemical oxidation of organic phosphorus 95

3.4.5 Comparison of manual SRP to automated SRP analysis 97

3.5 Test analysis of organic Phosphorus using ICP-MS and spectrophotometry 99

3.6 Discussion 101

3.6.1 Conclusions 103

Chapter 4: Dissolved organic phosphorus dynamics in the Christchurch Harbour system. 104

4.1 Introduction 104

4.2 Materials and Methods 106

4.2.1 Estuarine Transect sampling 107

4.3 Results 108

4.3.1 Total dissolved phosphorus (TDP) 108

4.3.2 Soluble Reactive Phosphorus (SRP) 112

4.3.3 Dissolved organic phosphorus (DOP) 115

4.3.4 Fluxes of Phosphorus 122

4.3.5 Relationships between phosphorus and environmental parameters 125

4.3.6 Estuarine transects 127

4.4 Discussion 133

4.4.1 Seasonal nutrient dynamics 133

4.4.2 Estuary processes 139

4.4.3 High flow events 141

4.4.4 EU Water Framework directive 144

4.4.5 Conclusions 146

Chapter 5: Summary and conclusions 148

5.1 Synthesis of results 148

5.2 Future perspectives. 150

Appendix 1 Relationships between phosphorus fractions and environmental parameters 152

Appendix 2 Relationships between inorganic nutrients and environmental parameters 168

Appendix 3 Relationships between river flux and inorganic nutrients 184

List of References 186

List of figures

Figure 1.1: Schematic diagram of the aquatic phosphorus cycle. From Worsfold et al., (2016)....3

Figure 1.2: Operationally defined P fractions. excluding particulate phosphorus. Adapted from Worsfold et al., (2005). 7

Figure 1.3: Location of the sample sites in relation to the UK. 18

Figure 2.1: Sonde measurements taken on the same dates as the water samples at Knapp Mill on the River Avon 32

Figure 2.2: Sonde measurements taken on the same dates as the water samples at Throop on the River Stour 33

Figure 2.3: Sonde measurements taken on the same dates as the water samples at Iford Bridge on the River Stour 34

Figure 2.4: Sonde measurements taken on the same dates as the water samples at Mudeford Quay at Christchurch Harbour. 35

Figure 2.5: Nitrate concentrations and river flow rate at Knapp Mill (A), Throop (B), Iford (C) and Mudeford (D). Error bars show +- 1 sd from 3 analytical measurements 40

Figure 2.6: Nitrite concentrations and river flow rate at Knapp Mill (A), Throop (B), Iford (C) and Mudeford (D). Error bars show +- 1 sd from 3 analytical measurements 41

Figure 2.7: Phosphate concentrations and river flow rate at Knapp Mill (A), Throop (B), Iford (C) and Mudeford (D). Error bars show +- 1 sd after 3 analytical repeats. 43

Figure 2.8: Silica concentrations and river flow rate at Knapp Mill (A), Throop (B), Iford (C) and Mudeford (D). Error bars show +- 1 sd after 3 analytical repeats. 45

Figure 2.9: Flux of nitrate (A), nitrite (B), phosphate (C) and silica (D) at Knapp Mill on the River Avon for the duration of the sampling period. 49

Figure 2.10: Flux of nitrate (A), nitrite (B), phosphate (C) and silica (D) at Throop on the River Stour for the duration of the sampling period 50

Figure 2.11: Mean daily flow rate, phosphate concentration and nitrate concentration at Knapp Mill on the river Avon from 1/1/00 to 1/10/15 from the Environmental Agency's routine sampling. For flow rate, the red points indicate the flow rate during the period of this investigation (April 2013-April 2014), for nitrate and phosphate, the red values indicate the concentrations derived from the inorganic analysis at the University of Portsmouth. 53

Figure 2.12: Mean daily flow rate, phosphate concentration and nitrate concentration at Throop on the river Stour from 1/1/00 to 1/10/15 from the Environmental Agency's

routine sampling. For flow rate, the red points indicate the flow rate during the period of this investigation (April 2013-April 2014), for nitrate and phosphate, the red values indicate the concentrations derived from the inorganic analysis at the University of Portsmouth. 54

Figure 3.1: Inorganic standard curve (A), Standard addition of 1ppb of Avon river water (B) and standard addition of 2ppb for Stour river water (C). 76

Figure 3.2: Standard curve comparison between phosphoric acid, phytic acid and glycerophosphate (GLP). 78

Figure 3.3: Standard curves of glycerophosphate (GLP) and phytic acid compared to a 1:1 line 78

Figure 3.4: Analysed CRM concentration compared to the gravimetrically predicted CRM concentration and the SRP concentration of the CRM analysed using inorganic spectrophotometry. Error bars show 1 sd. 80

Figure 3.5: Total phosphorus concentration of samples following freezing and filtration. Error bars show 1sd. Each sample and blank was replicated 5 times. 84

Figure 3.6: TDP and SRP of a filtered and unfiltered sample. Each sample was replicated 4 times. Error bars show ± 1 sd. 85

Figure 3.7: Phosphorus concentrations of digested and undigested filtered water samples analysed using ICP-MS and the SRP by spectrophotometer. Error bars show ±1sd. 87

Figure 3.8: Absorbance at 885nm of filtered and unfiltered water samples and Milli-Q blanks. Each sample and blank was replicated 10 times with error bars representing +/- 1 standard deviation. 91

Figure 3.9: Standard curves from 5 sets of analysis of SRP with absorption measured at 885nm on a spectrophotometer. Each standard curve was created using similar standard dilutions by weight – see example in table 3.3. 92

Figure 3.10: SRP standard curves using a 4cm pathlength cuvette (A and C) and 1cm pathlength cuvettes (B and D) A and B show the entire standard curve up to 45µM PO_4, C and D show the standard curve up to 20µM PO_4. Standards were analysed as single replicates. 94

Figure 3.11: DOP concentration determined by subtraction of manually analysed SRP from TDP derived from chemical oxidation. Weekly collected samples were analysed from 24 dates from 4/7/13 to 9/12/13 (o and * show outliers). 96

Figure 3.12: SRP concentration comparison between analysis done at the University of Portsmouth on an Autoanalyser and the manual SRP analysis from this investigation. Line = 1:1 98

Figure 3.13: SRP (light grey) and TDP concentrations (dark grey) derived from and spectrophotometric analysis at 885nm and HR-ICP-MS analysis of 4 samples from the River Avon. Error bars show +/- 1sd. Each sample was replicated 3 times. 100

Figure 4.1: Location of the riverine and estuarine transect sites. Site 1 is Mudeford Quay, 2 is the Ferry Pontoon, 3 is Blackberry Point, is Grimbury Marsh, 5 is Christchurch Quay, 6 is Tuckton Bridge 107

Figure 4.2: Total dissolved phosphorus (TDP) concentration at Knapp Mill (A), Throop (B), Iford (C) and Mudeford Quay (D). Error bars show ± 1 sd of 3 replicates. The line shows the daily mean river flow rate at each of the sites. The combined flow rate of the River Avon at Knapp Mill and the River Stour at Throop are used for Figure D. 111

Figure 4.3: Soluble reactive phosphorus (SRP) concentration at Knapp Mill (A), Throop (B), Iford (C) and Mudeford Quay (D). Error bars show ± 1 sd of 3 replicates. The line shows the daily mean river flow rate at each of the sites. The combined flow rate of the River Avon at Knapp Mill and the River Stour at Throop are used for Figure D. 114

Figure 4.4: Dissolved organic phosphorus (DOP) concentration at Knapp Mill (A), Throop (B), Iford (C) and Mudeford Quay (D). Error bars show ± 1 sd of 3 replicates. Points where the DOP concentration was below detection limits are indicated by an *. The line shows the daily mean river flow rate at the sites. The combined flow rate at Knapp Mill and at Throop are used for Figure D... 117

Figure 4.5: Dissolved organic phosphorus % (DOP%) at Knapp Mill (A), Throop (B), Iford (C) and Mudeford Quay (D). Error bars show ± 1 sd of 3 replicates. The line shows the daily mean river flow rate at each of the sites. The combined flow rate of the River Avon at Knapp Mill and the River Stour at Throop are used for Figure D. 121

Figure 4.6: Fluxes of SRP, TDP and DOP from Knapp Mill on River Avon (left panels) and Throop on River Stour (right panels) over the sampling period. River flow is shown from the gauging stations at Throop and Knapp Mill 124

Figure 4.7: Concentrations of TDP (left column), SRP (middle column) and DOP (right column) at the sampling points from the transect analysis (Figure 2) on the 5 dates in which transect analyses were done. Grey bars show P concentration in µM and red lines show salinity. Error bars show 1 sd after 3 replicates. 129

Figure 4.8: Relationships between SRP (left plots), TDP (right plots) and salinity at sites 1-6 on the 5 dates in which transect samples were analysed. Lines indicate mixing between maximum concentrations at zero salinity and minimum P concentrations at maximum salinity values 131

Figure 4.9: Relationships between DOP (left plots), DOP % (Right plots) and salinity at sites 1-6 on the 5 dates in which transect samples were analysed. Lines indicate mixing between maximum concentrations at zero salinity and minimum P concentrations at maximum salinity values. 132

List of tables

Table 2.1: Detection limits for the multiparameter sondes used in this investigation 27

Table 2.2: Minimum, maximum, mean and standard deviation of the measurements taken from the YSI EXO 2 Sonde and river flow data from the environment agency for the duration of the sampling period. 37

Table 2.3: Minimum, maximum, mean and standard deviation of the inorganic nutrients for the duration of the sampling period 38

Table 2.4: Boundaries for water quality classifications as described by the EU Water Framework Directive (2000). The River Stour and Avon both fall into the 'Lowland', 'high alkalinity' category. 59

Table 3.1: Typical Order of HR-ICP-MS Analysis in a measurement period................................ 72

Table 3.2: Acquisition parameters for the HR-ICP-MS method used in this investigation.......... 72

Table 3.3: Dilutions required to create the inorganic standard curve for ICP-MS analysis......... 74

Table 3.4: Concentration of a water sample from the River Avon and the River Stour derived from a standard addition and an external, inorganic standard curve. 75

Table 3.5: Preparation of a CRM standard by dilutions from 2090 ppm to 100ppb. 79

Table 3.6: Gravimetrically derived P and ICP-MS analysed P in a CRM in 5 repeated samples. . 81

Table 3.7: Dilutions required to create the inorganic standard curve for colourimetric SRP analysis.. 89

Table 4.1: Maximum, minimum, mean and standard deviation of the SRP, TDP and DOP concentration at the four sites from 26/4/13 to 10/8/14. 50 samples were analysed at Knapp Mill, Throop and Mudeford and 40 at Iford. BDL= below detection limit (0.02µM). 109

Table 4.2: Relationships between, TDP, SRP and DOP and physiochemical parameters (Appendix 1). All parameters were measured using a YSI EXO 2 sonde with the exception of flow rate which measured by the Environment agency. Any values highlighted in red are significant (p-value<0.05) 126

Table 4.3: Concentrations of SRP, TDP and DOP at the riverine transect sites 127

Table 4.4: TDP, SRP and DOP concentrations at a number of riverine sites from this current investigation and other studies. 137

Table 4.5: TDP, SRP and DOP concentrations at a number of riverine sites from this current investigation and other studies. 138

Table 4.6: Proportion of samples, which were categorised as poor, moderate, good of high with regard to the EU Water Framework Directive, CEC (2000) 144

Table 4.7: Boundaries for water quality classifications as described by the EU Water Framework Directive (2000). The River Stour and Avon both fall into the ‘Lowland’, ‘high alkalinity’ category. 145

Declaration of authorship

I, JACK BILLINGE declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

DISSOLVED ORGANIC PHOSPHORUS CONCENTRATIONS IN A RIVER AND ESTUARY SYSTEM

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. [Delete as appropriate] None of this work has been published before submission [or] Parts of this work have been published as: [please list references below]:

Signed: ....

Date: ....

Acknowledgements

I would first like to thank my supervisors, Professor Duncan Purdie, Professor Martin Palmer, and Dr Andy Milton for their help and support throughout the PhD process.

Special thanks goes to other members of the Christchurch Harbour Macronutrients consortium, particularly Anouska Panton and Jiraporn Charoenvattanaporn.

Thanks must also go to Dr Matt Cooper, Agnes Michalik, and Matt O'Shaughnessy, for all their help with lab equipment, general lab queries and useful advice.

I also have to give thanks to my friends and family for their continued support, especially Tom and Lou.

To Mum and Colin, Matt, Kath and Rich for always being there. To Alice and Andy, for letting me gatecrash your house so soon after getting married. To Finola for keeping me going throughout and to Dad for getting me interested in science in the first place.

Definitions and Abbreviation

ATP	Adenosine tri-phosphate
CHMC	Christchurch Harbour Macronutrients Consortium
CRM	Certified reference material
DOP	Dissolved organic phosphorus
DNA	Deoxyribose nucleic acid
FNU	Formazin Nephelometric Unit
EA	Environment Agency
HDPE	High density polyethylene
ICP-MS	Inductively coupled plasma—mass spectrometry
N	Nitrogen
NOCS	National oceanography centre, Southampton
NTU	Nephelometric Turbidity Unit
P	Phosphorus
PPE	poly-phenylene ether
RNA	Ribose nucleic acid
Si	Silica
SRP	Soluble reactive phosphorus
TP	Total phosphorus
TDP	Total dissolved phosphorus
TOP	Total organic phosphorus
TRP	Total reactive phosphorus
WFD	EU Water Framework Directive

Chapter 1: Introduction

Nitrogen (N), Phosphorus (P), and Silicon (Si) are macronutrients, vital for the metabolism and growth of many organisms in rivers, estuarine and coastal waters. Riverine inputs are a major source of these macronutrients to coastal zones via estuarine systems. For example, Chinese rivers deliver approximately 5-10% of global freshwater input to the world ocean and 15-20% of the global continental sediment with associated nutrient loads (Liu et al., 2009). However, the fluxes of macronutrients from rivers to coastal areas via estuaries is complex and accurate knowledge of the hydrodynamics and the biogeochemical processes in estuarine systems is key (Statham, 2012).

Eutrophication is the process in which a water body becomes enriched in nutrients, particularly nitrates and phosphates. This causes a consequent increase in growth of photosynthetic plants and algae which then die and decompose. The microbial decomposition of this organic matter depletes the oxygen in the water body and increases the concentration of carbon dioxide leading to anoxic conditions, causing losses of biodiversity and large scale ‘dead zones’ (Diaz et al., 2008a).

A primary cause of eutrophication is the intensification of agriculture globally (Matson et al., 1997). In 1950, global production of agricultural fertilisers released more than 10 million tonnes of nitrogen into rivers and this may exceed 135 million tonnes by the year 2030 (Vitousek et al., 1997). Substantial amounts of nitrates and phosphates are also applied to agricultural areas in the form of animal manures.

In addition to the diffuse agricultural inputs of nitrate and phosphate, sewage treatment work effluent inputs into rivers can pose greater risks for river eutrophication than the agricultural inputs (Jarvie et al., 2006). Reducing the delivery of these major nutrients into rivers and estuaries is often considered the principal means of delivering improved ecological status of rivers. It is vital in complying with legislation such as the EU Water Framework Directive (CEC, 2000) and other international criteria.

1.1 Phosphorus

Phosphorus is a key element for life on Earth and controls many biogeochemical processes in freshwater and marine systems. Phosphorus provides the phosphate-ester backbone of DNA and RNA, and is critical in the transmission of chemical energy using the ATP molecule as well as being a structural constituent in many cell components such as phospholipid cell membranes.

Phosphorus is present in both organic and inorganic forms in aquatic and marine environments. A non-renewable mineral, phosphorus is the 11th most abundant element in the Earth's crust, approximately 0.1% by mass (Paytan and McLaughlin, 2007). The most common P containing mineral in the Earth's crust is apatite, containing 95% of P. However, phosphate (PO_4) has been described in approximately 300 additional minerals. Inorganic phosphate in aquatic and marine environments is dominated by orthophosphoric acid that is protonated to varying degrees depending on the ionic composition of the water (Statham, 2012). Polymeric forms of inorganic P are also found in generally low concentrations but can constitute up to 7% of total P in surface waters during phytoplankton blooms (Diaz et al., 2008). As P is a vital component of cellular structures, such as ATP, organic forms of P can also be found in the marine environment. Phosphorus is also present as phosphine gas (PH_3), a highly toxic and reducing gas which is ubiquitous in the Earth's atmosphere, mainly produced in wetland areas like lakes and paddy fields (Han et al., 2011), as well as in selected anoxic marine habitats (Karl and Bjorkman, 2002). Despite the atmospheric inputs of P, the main source of P to the oceans is riverine influx (Benitez-Nelson, 2000). Figure 1.1 provides a schematic diagram of the aquatic phosphorus cycle, showing the sources, sinks and fluxes.

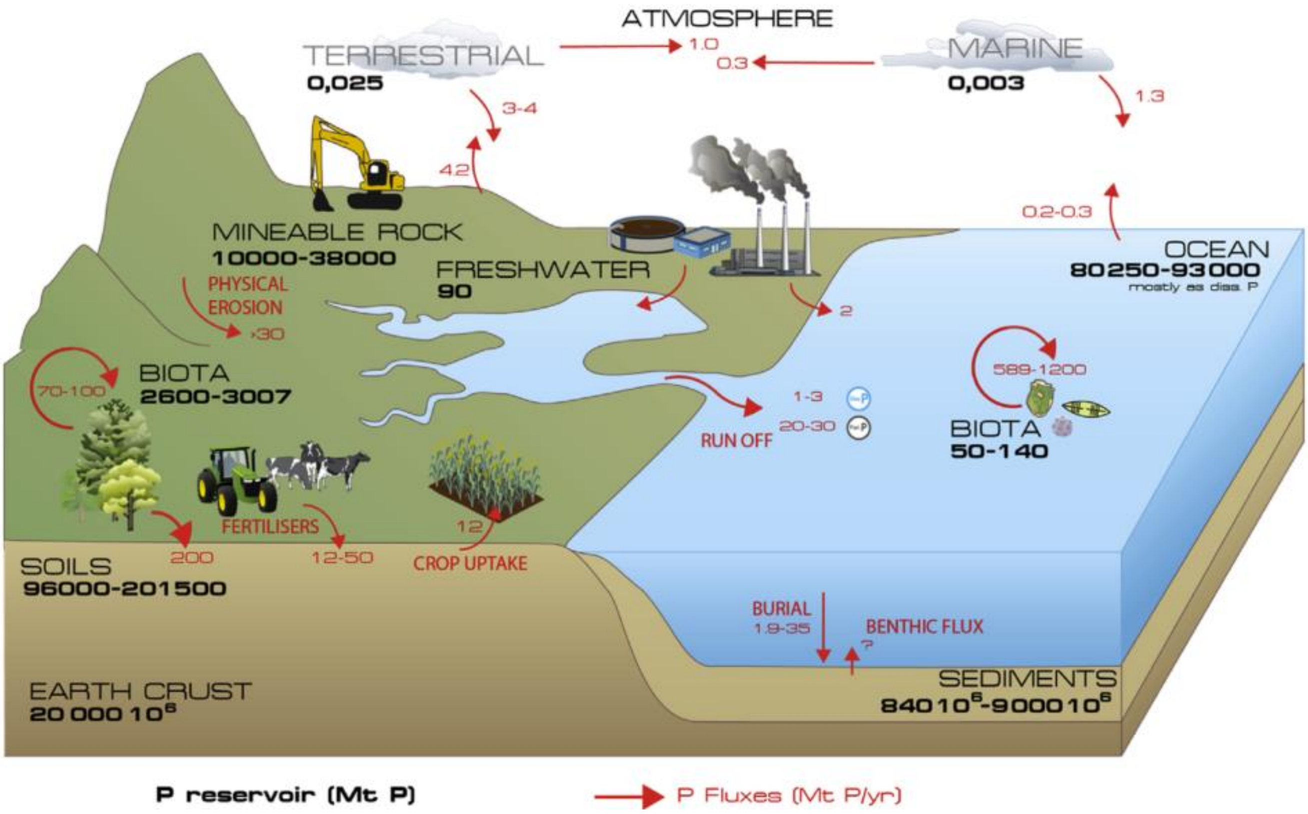


Figure 1.1: Schematic diagram of the aquatic phosphorus cycle. From Worsfold et al., (2016).

Anthropogenic phosphorus enters rivers and estuaries via land run off after application of fertilisers (Carpenter et al., 1998), from point sources such as sewage effluent (Jarvie et al., 2006), septic tank effluent (Zurawsky et al., 2004) and from the release into rivers of chemicals such as detergents (Richards et al., 2015). The application of P based fertilisers has made intensive production of human foodstuff possible and is critical in farming around the globe. However, leaching of the fertilisers into aquatic systems can lead to eutrophication and increase the frequency of harmful algal blooms in coastal areas (Tilman et al., 2001).

In addition to this, point source inputs of sewage effluent into rivers can be a larger source of P than the diffuse agricultural inputs (Jarvie et al., 2006) and it has been estimated that the river-derived global transport of dissolved and particulate P are 32–58 and 590–650×10^9 mol yr^{-1} respectively (Ruttenberg, 2003). Phosphorus fluxes to the ocean in the UK remain dominated by P inputs from urban areas, despite the introduction of legislation such as the EU Waste Water Treatment Directive, which initiated enhanced P stripping from effluent (Worrall et al., 2016). In the UK, there are no significant indigenous phosphate rock resources, with the majority of current production being concentrated in a handful of countries such as China and Morocco (Cooper et al., 2011). This means that the UK imports large amounts of phosphorus-based fertilisers, which has caused a net accumulation of P over the last 15 years at a rate of 0.6 ktonnes P/year across the whole of the UK, equivalent to (24 kg P/km^2/year^2) (Worrall et al., 2016). The detrimental impact of elevated phosphorus concentrations in natural waters has led to the inclusion of phosphorus standards in numerous legislative frameworks and guidelines globally. In Europe, the Water Framework Directive (WFD) (WFD: CEC, 2000) provides standards for river basins, estuaries and coastal margins and the Marine Strategy Framework Directive (MSFD, 2008) covers marine water. The UK Technical advisory group on the Water Framework Directive (UKTAG) provides discussion of the WFD with relation to UK phosphorus standards in natural waters (UKTAG, 2008; UKTAG, 2013). Updated standards from the 2013 report (UKTAG, 2013) builds on improvements in understanding of the relationships between river plant communities and elevated phosphorus concentrations, derived from a “new approach” to setting phosphorus standards that produces site-specific estimates of natural phosphorus concentrations, taking account of a site’s alkalinity and altitude. The WFD categorises natural waters as “High”, “Good”, “Poor” or “Bad” with regard to phosphorus. Therefore, reliable measurements and an appropriate sampling frequency for phosphorus will be a prerequisite for further refinement of legislative guidelines for phosphorus (Bowes et al., 2015).

Phosphorus is present in a range of forms in the marine environment, however, the nomenclature used to distinguish between the forms can be confusing (Figure 1.2), so standardised nomenclature and analytical techniques are needed. In phosphate analysis, samples are often referred to as 'dissolved' or 'total' phosphorus. Usually, dissolved phosphorus is defined as the fraction of phosphorus that will pass through a 0.7µm, 0.45µm or 0.2µm filter. The total phosphorus fraction is any phosphorus analysed without the application of a filtration step. The dissolved or total fractions can then be analysed in a variety of ways to provide information on the chemical composition of the phosphorus fractions. A simplified version of this can be seen in Figure 1.2. The established method for the analysis of dissolved inorganic P is a modified molybdenum blue method described by Murphy and Riley (1958). This is a well-documented technique for the analysis of inorganic P, although it has been criticised for the possible hydrolysis of organic compounds due to the acidic nature of the assay (Baldwin, 2013). However, other studies have questioned the extent of this hydrolysis if the samples are analysed within 4 hours of the acidic addition (Denison et al., 1997). The concentration of inorganic phosphorus when the sample is analysed post filtration is often referred to as soluble reactive phosphorus (SRP) (Worsfold et al., 2005) and total reactive phosphorus (TRP) when analysed with no filtration step. In order to analyse the total phosphorus (TP) in a sample, an oxidation step is used, which converts organic forms of P to inorganic phosphate. If a sample is analysed this way after filtration the fraction is referred to as total dissolved phosphorus (TDP) and as TP when analysed without filtration. There are a number of techniques used as a step to convert particulate and dissolved organic P to inorganic phosphate. The most common technique is the addition of an oxidising agent to the sample such as perchloric acid, hydrogen peroxide, sulphuric acid-nitric acid or peroxydisulphate (Maher and Woo, 1998). In previous studies, addition of potassium persulphate with hydrochloric acid before a heating step is the most frequently used methodology. A heating step is used in most studies in order to increase the conversion of TP to SRP. The sample can be heated by microwave, autoclave or conventional heating. Ultraviolet photo-oxidation is another commonly used technique to determine total phosphorus content in a water sample

(Roig et al., 1999). However, the relative effectiveness of digestion methods is variable so the TP or TDP value will vary according to the rigour of the digestion method used (Jarvie et al., 2002). Subtraction of the SRP concentration from the total phosphorus concentration is then termed total dissolved phosphorus, if analysed without a filtration step or dissolved organic phosphorus if analysed after filtration (Worsfold et al., 2005; Rinker and Powell, 2006; Statham, 2012; Ruttenberg and Dyhrman, 2012; Van Moorleghem et al., 2013; Qin et al., 2015; Worsfold et al., 2016), with the caveat that SRP may already include some labile DOP (Baldwin, 2013).

Another method to analyse total phosphorus in water samples is inductively coupled plasma mass spectrometry (ICP-MS). ICP-MS as a technique to analyse TDP has not been widely implemented, but a select few studies have had success with the method. Cantarero et al., 2002, investigated TDP concentrations in agricultural run-off and obtained similar TDP concentrations with ICP-MS and using chemical oxidation methods. Ivanov et al., 2010 investigated soil and plant TP concentrations with ICP-MS after an acidic digestion stage. Results indicated that ICP-MS provided lower concentrations of TP than chemical oxidation methods, most likely due to matrix interferences. One of the aims of this PhD thesis was to develop an ICP-MS method for analysing phosphorus in a large number of environmental water samples.

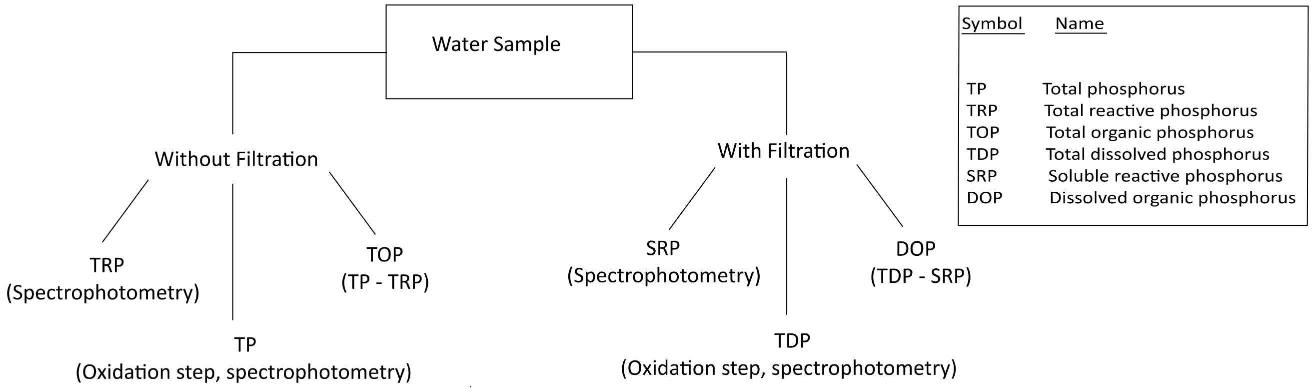


Figure 1.2: Operationally defined P fractions. excluding particulate phosphorus. Adapted from Worsfold et al., (2005).

One fraction of phosphorus which is understudied but biologically important is dissolved organic phosphorus (DOP). DOP in aquatic and marine environments is a diverse assemblage of compounds including nucleic acids, other nucleotides (e.g. ATP, GTP and AMP), inositol phosphates, phospholipids and phosphonates (Baldwin, 2013). Esters are readily hydrolysed by bacterial and phytoplankton alkaline phosphatases (Chrost and Overbeck, 1987), and increases in phytoplankton phosphatase activity have been used as an indicator of SRP stress (Hoppe, 2003; Dyhrman and Ruttenberg, 2006). DOP has been observed to decrease the SRP demand of the phytoplankton community and is an important source of phosphorus for autotrophs (Dyhrman and Ruttenberg, 2006, Nausch and Nausch, 2007, Rotner et al., 2016), particularly in oligotrophic oceanic environments (Lomas et al., 2010, Dyhrman et al, 2006, Sebastián et al., 2012). In addition to this, in aquatic environments enriched in iron (oxyhydr)oxides, DOP bioavailability may exceed that of orthophosphate due to different sorption rates of SRP and DOP (Ruttenberg and Sulak, 2011).

DOP often accounts for over 80% of the TDP pool in the North Atlantic Ocean (Wu et al., 2000; Ammerman et al., 2003; Lomas et al., 2010) and has been recorded at over 75% in rivers (Neal et al., 2000a). Despite the increasing evidence for the importance of DOP utilization in marine and aquatic environments, relatively little is known about the seasonal variability in DOP concentrations in river and estuary systems. Monbet et al., (2009) analysed water samples collected from 8 stations from riverine to marine end-members on the River Tamar in Devon UK during four seasonal campaigns. DOP was found to comprise 6-40% of the TDP with the highest and lowest concentrations observed in autumn and summer, respectively. In addition to this, the total enzymatically hydrolysable phosphorus (EHP) pool, which consists of the whole DOP fraction hydrolyzed by the combination of three enzymes, was recorded. This labile portion of the total DOP constituted 6-40% of the DOP and behaved non-conservatively along a salinity gradient in the Tamar Estuary (Monbet et al., 2009)

The dissolved organic fraction (DOP) can be obtained by subtraction of the SRP concentration from the TDP concentration. The analytical method utilised varies but usually requires that a sample must first be digested to convert all organic and colloidal P forms to detectable inorganic phosphate before analysis. There are a number of techniques available as described above for the digestion of TDP to SRP. The most commonly used chemical technique is by the addition of an oxidising agent, such as potassium perchlorate, in an acidic or alkaline medium, at high temperature. However, ICP-MS is an emerging technique for TDP analysis (Ivanov et al., 2010, Regelink et al., 2013) and may have significant advantages over previous chemical techniques. For instance, pre-analysis with HPLC (Heerboth, 2007; Atlas et al., 2015), or field flow fractionation (Regelink et al., 2013) on-line using ICP-MS is possible. However, a robust methodology for analysis of TDP using ICP-MS has not been described prior to this study.

1.2 Nitrogen

Nitrogen (N) is required in the synthesis of amino acids, nucleic acids and is an important component of chlorophyll, making it vital for photosynthesis. The nitrogen cycle therefore affects the phosphorus and carbon cycle as well as other global elemental cycles.

Both the oxidized and reduced inorganic N species of nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+), ammonia (NH_3) and organic N fractions of dissolved organic nitrogen (DON) and particulate organic nitrogen (PON) are commonly present in all freshwater, estuarine and coastal waters. Nitrogen speciation in rivers varies with N enrichment. In highly enriched rivers nitrate is the dominant form, whereas DON is the dominant form in less enriched rivers (Durand et al., 2011). Most data reported for nitrogen are for inorganic nitrate as it is the most dominant form under oxic conditions as well as being the N species most associated with eutrophication (Statham, 2012).

Nitrite is typically found in much lower concentrations than nitrate, but is available to a wide range of aquatic organisms (Dham et al., 2005, Martiny et al., 2009). Nitrite is formed in aerobic conditions by ammonium oxidising microbes (Olson, 1981) or from incomplete assimilatory reduction of nitrate by phytoplankton (Collos, 1998) and bacteria (Wada and Hattori 1971, Lomas and Lipschultz, 2006). In addition to nitrate and nitrite, anaerobic oxidation of ammonia (annamox) converts nitrite and ammonium to diatomic nitrogen and water, and recent studies have shown that it can contribute up to 58% of in situ nitrogen gas production in riverbeds (Lansdown et al., 2016).

Organic forms of N are difficult to quantify in river and estuarine systems due to the multitude of compounds present, from small bioavailable compounds such as amino acids to larger molecular weight polymeric substances which are more resistant to decomposition. DON is generally regarded as a small fraction of the total nitrogen pool, however in some rivers the DON % can be up to 80% of the total combined nitrogen (Magalhaes et al 2008). DON can provide significant proportions of total N demand in conditions of low inorganic nitrate availability (Zhang et al., 2015). The DON concentration frequently exceeds the inorganic nitrate concentration in marine and fresh waters (Stepanauskas et al., 2002; Berman and Bronk, 2003).

Nitrogen reaches freshwaters via a number of pathways. Leaching and run off from diffuse sources such as from land which has had fertiliser applied is a major contributor. Also, point source inputs from sewage treatment works (Jarvie et al., 2003) can contribute large loadings of total N into riverine systems. Bowes et al, (2015) found that nitrate inputs into the river Enborne had large groundwater inputs mainly due to the chalk-based catchment, in addition to effluent from sewage treatment works and urban run-off.

Nitrogen occurs in many combined forms in the aquatic environment including ammonia, nitrate, nitrite, and organic each of which can be measured in a variety of ways. These methods include volumetric analysis, spectrometry, electrophoresis and liquid chromatography. However, the most widely used analytical technique for nitrate analysis is via an initial reduction to nitrite using cadmium metal, giving a total oxidisable N concentration. Nitrite is then analysed colourimetrically by diazotization with sulfanilamide and subsequent coupling with N-(1-naphthyl) ethylenediamine which forms a coloured azo-dye. This dye is analysed spectrophotometrically at 543nm and the difference between the total oxidisable N and the nitrite gives the nitrate concentration.

1.3 Silicon

Silicon is the second most abundant element in the Earth's crust, but only a minor portion takes part in biogeochemical cycles. The biogeochemical cycle of silica depends on the weathering of rocks releasing dissolved silicon into rivers. Due to this, riverine inputs of silica to coastal zones vary on geological time scales (White and Blum, 1995), with large scale geological events, such as the uplift of the Himalayan Plateau having an impact by exposing new rock (Raymo, 1991). Despite this, anthropogenic impacts such as damming, deforestation and the introduction of invasive species are affecting the delivery of dissolved silica into rivers and oceans (Conley et al., 1993; Humborg et al., 2000; Ragueneau et al., 2005).

Dissolved silicon or biogenic silica, is utilised by diatoms and other siliceous organisms such as radiolarians and silicoflagellates, which are strictly dependent on dissolved silica to build their biomineralised cell walls. Diatoms are the most important group of planktonic algae in terms of biomass in the majority of marine ecosystems accounting for 60% of the world's primary production (Tréguer et al., 1995). The availability of biogenic silica in the ocean is a major control over these silicaceous organisms. The availability of dissolved silicon can be the major limiting nutrient in rivers and oceans, depending on the availability of other nutrients such as N and P (Bernard et al., 2009). The availability of dissolved silica can have an influence on phytoplankton as well as on primary production. Most diatoms require dissolved silicon in the amount of roughly 1 mol for every mole of N assimilated (Howarth et al., 2011). If dissolved silicon becomes scarce, diatoms can be replaced by other phytoplankton species with higher growth rates that do not require dissolved silicon.

The standard method for analysis of dissolved silicon in water samples involves the formation of a silicomolybdate complex (Strickland and Parsons, 1968) that is then reduced, using oxalic acid, to produce a blue solution that has an absorption maximum at 810 nm wavelength, which is proportional to the colour's intensity.

1.4 Estuaries

Estuaries and coastal zones are focal points for human settlement, with approximately 40% of the global population living within 100km of the coast (SEDAC, 2011). They have a wide range of forms and there are over 40 different definitions of estuaries (Perillo, 1995). However, the most accepted definition is that "an estuary is a semi-enclosed coastal body of water which has a free connection to the open sea and within which sea water is measurably diluted with fresh water derived from land drainage" (Cameron and Pritchard, 1963). Due to the mixing of the saline and fresh water, estuaries provide a challenging habitat and they are home to a wide range of unique organisms.

Rivers are the main pathway of nutrients and pollutants to the ocean via estuaries (Sharples et al., 2017). The transformation of nutrients in estuaries is complex and urbanised estuaries are often prone to contamination from both point and diffuse sources. In terms of water exchange, tidally driven fluxes of seawater often greatly exceed freshwater inputs. However, river water typically will have very much greater concentrations of nutrients than the adjacent coastal seawater, causing a net flux from land to sea (Statham, 2013) but the fluxes of nutrients through estuaries are also impacted by a combination of numerous hydrodynamic and biological processes (Nedwell et al., 1999; Statham, 2013) . The fluxes of nutrients in estuaries are also closely linked to the geography of the estuary, which means that any human impact on the geology of a river, such as reclaiming wetlands, can cause changes to the nutrient fluxes in estuarine systems (Jickells et al., 2014)

The hydrodynamics of estuarys involves a range of processes including circulation and mixing due to both freshwater and saline water inputs, heat input during high insolation periods as well as the Coriolis effect, that affects large estuaries (Huijts et al., 2006; Statham, 2013). These factors lead to varying degrees of stratification and estuaries can be classified with respect to the type and level of mixing. These classifications include well-mixed estuaries, partially mixed estuaries and highly stratified estuaries, with other classifications such as salt-wedge and vertically mixed estuaries being used. Fjords are formed when an advancing glacier carves out a deep valley and leaves a sill at the mouth of the estuary caused by the glaciers reduced erosion rate and deposition of terminal moraine. These topographic effects can lead to reduced circulation and extended periods of anoxia with periodic large flushing events. One of the main hydrodynamic features of estuarine systems is the flushing time, which is the time needed to replace the freshwater volume of a system at the rate of net flow through the estuary. Flushing time can vary in the same estuary at different times of the year. McLusky and Elliot (2004) state that the flushing time of the Forth estuary under mean river flow conditions was 12 days, but increased to 10 weeks during low river flow periods and decreased to 6 days during periods of high river flow. Flushing times also vary from hours and minutes in very small estuaries, to weeks and months in larger estuaries. Flushing time can have an important impact on the biology of estuaries as it determines the amount of time an organism is exposed to a pollutant and can influence the net growth of estuarine phytoplankton and zooplankton populations . Additionally, the longer the flushing time, the higher the chance of exchanges across the benthic sediment/water interface and the higher chance that biological activity will affect the biogeochemistry of the water column (Statham, 2013; Human et al., 2015).

Water enters rivers and estuaries from run-off from land but in some systems can also be groundwater fed. It has been estimated that the total flux from submarine groundwater discharge could be between 80 and 160% of the amount of freshwater entering the Atlantic Ocean from rivers (Moore et al., 2008). The flow of water between groundwater and surface water occurs at different spatial and temporal scales (Malard et al., 2002; Pretty et al., 2006). On a large scale, catchment geology, land use and hydrology affect groundwater-surface water exchange; whereas on a small scale, channel morphology and flow dynamics play a role (Malard et al.,2002). Contamination of groundwater is a growing environmental problem, and it has been estimated that diffuse agricultural activities are responsible for 70-80% of nitrate in English surface and groundwaters (Defra, 2002).

Estuaries generally have high concentrations of suspended particulate matter (SPM) compared to coastal waters and play an important role in biogeochemical processes within estuaries (Turner and Millward, 2002). Estuarine SPM is derived mainly from river inputs, resuspension or import from coastal waters, with a minor atmospheric input (Statham, 2013). Transfer of suspended particles through estuaries tends to be non-conservative, and riverine SPM is extensively modified in tidal estuary systems before it is transferred to the sea (Middelburg and Herman, 2007). Factors affecting the formation and breakup of suspended particle aggregates within estuary systems include the intensity and scale of turbulent shear, differential settling of particles, the concentration of SPM, Brownian motion and salinity as well as the presence and concentration of sticky polymers derived from biota (Fugate and Friedrichs, 2003). These particles provide a surface area which can interact with various constituents of the water column, including trace metals (Wang and Liu, 2003). The presence and concentration of SPM within an estuarine system can also affect the attenuation of light, which can have effects on phytoplankton production and community structure within the system (Irigoien and Castel, 1997).

The behaviour of elements in estuaries can be described as conservative or non-conservative. If there is no removal within the estuarine system, the concentration of nutrients in river water when diluted with saline water will decrease linearly enabling predictions of concentrations by simple models. However, if there is removal or addition of nutrients in the estuary, the relationship between concentration and salinity will not be linear causing difficulty in making predictions. Due to a combination of urbanisation, industrialisation and changes in land use practices, the fluxes of many nutrients into estuarine systems is increasing (Bowen and Valiela, 2001; Nedwell et al, 2002). For example, between 1960 and 1998 the nitrogen flux to the Mississippi basin increased by 80% with a higher proportion of nitrates present (McIsaac et al., 2001). Globally rivers deliver large quantities of N, P and Si the coastal ocean, via estuaries, each year (Sharples et al, 2017) and are estimated to deliver 4-11 Tg total phosphorus yr^{-1} (*Seitzinger et al*., 2005), 37–66 Tg total nitrogen yr^{-1} and 340–380 Tg dissolved Si yr^{-1} *(Treguer et al*., 1995; *Beusen et al*., 2009), which can cause increases in oceanic biological activity. Nedwell et al, (2002) studied nutrient loads in every UK mainland estuary and found significant correlations between chlorophyll a concentrations in coastal waters and the log total annual loads of total oxidisable nitrate, ammonium and phosphate for each adjacent estuary.

Phosphorus can be significantly modified within an estuarine system with particle-water exchanges either increasing or reducing the flux of SRP from estuaries to coastal seas

(Prastka et al., 1998). Phosphorus is present in the range of fractions described in Figure 1.2, as well as in particulate and colloidal forms. Cai and Guo (2009) used ultrafiltration to investigate the partitioning of phosphorus between dissolved, colloidal and particulate phases in an estuarine system. They found that dissolved inorganic phosphorus was the dominant species in estuarine and marine waters whereas dissolved organic phosphorus was the dominant species in river water Their investigation, based on a number of river systems in North America showed that the colloidal fraction varied between the rivers, from 41-88%, indicating that the colloidal fraction in river and estuarine systems is affected by terrestrial inputs, autochthonous production, and anthropogenic activities in the river and catchment area system. The partitioning of phosphorus in estuarine systems is complex and related to the hydrodynamics of the estuarine system. Liu et al., (2011) found that in the whole river system, particulate P was the dominant species, with dissolved P being second and colloidal P being the least dominant form. Nitrogen, mainly nitrate, can undergo significant denitrification within estuaries, reducing nutrient inputs from rivers to the sea (Loken et al., 2016). A study by Silvennoinen et al., (2007) found that in estuaries with small residence times, denitrification removed up to 9% of the nitrate load and up to 19% in an estuary with high residence times, confirming previous studies that found a positive relationship between residence time and nutrient transformation (Dettman, 2001).

Often denitrification and nitrification are linked and may be affected by eutrophication (Risgaard-Petersen, 2003). In addition to this, gaseous forms of N (nitrogen and nitrous oxides) may be important in losses of N from estuarine systems (Statham, 2012) and organic forms of N are also present in estuaries.

Dissolved silica concentrations in estuaries can be linked to changes in anthropogenic interference of sedimentation, for example damming upstream of freshwater reduces the flux of dissolved silica into coastal areas (Humborg et al., 2000).

The complex processing of nutrients in estuaries creates problems for global nutrient flux models (Sharples et al., 2016), as the behaviour of nutrients in estuaries is not well understood. However, it has been suggested that 30–65% of N and 10–55% of P is removed inside estuaries before release to the coastal ocean (Nixon et al., 1996). In order for effective modelling of estuarine environments, an understanding of the key processes and interactions is vital (Statham, 2013). Currently, research is focussing on high-resolution in situ sampling to analyse nutrients (Yates and Johnes, 2013; Bowes et al., 2015). This is needed in order to identify episodic high-intensity events, such as storms, and the effect that they have on nutrient flux. The role of organic forms of N and P are also poorly understood in estuarine systems. DOP for example has been shown to be bioavailable and can form up to 40% of the total P pool in estuaries (Monbet et al, 2009).

1.5 Research area

A frequent set of measurements of nutrients have been made over an annual period at sites in the lower reaches of the Hampshire Avon river, Dorset Stour river and Christchurch Harbour estuary (Figure 1.3).

The River Avon is 96km long with a catchment area of approximately 1750km^2 containing 2 areas of outstanding natural beauty, 2 environmentally sensitive areas, 9 special areas of conservation, 6 national natural reserves and 71 sites of special scientific interest. The population of the catchment stands at 230 000 and it is only 2% urbanised. The main urban areas in this catchment are Salisbury, Christchurch and Warminster. Rural land use accounts for 98% of the area and comprises of woodland, arable and improved pasture grasslands (CaBA 2016). The Hampshire Avon is fed from groundwater from Cretaceous chalk and upper greensand aquifers. It has also been flagged as a river of national importance due to its predominantly agricultural watershed and elevated inorganic nutrient loadings (mean nitrate concentration ≈ 400 µM; Jarvie et al., 2005). The Hampshire Avon was chosen to be the focus of this study for a variety of reasons, including the fact that it is currently the focus of a number of large research projects; the whole catchment is part of a DEFRA sponsored Demonstration Test Catchment project that aims to assess the effectiveness of on-farm mitigation measures for reducing diffuse pollution from agriculture to water and its impacts on aquatic ecology, in order to maintain a working agricultural sector. In addition to this, the Hampshire Avon also forms part of the 'Catchment Sensitive Farming Initiative' (CSF), with the aim of identifying ways to reduce agricultural-based pollution using on-farm advice and voluntary works, in conjunction with some capital funded items such as those targeting farm infrastructure upgrades (DEFRA, 2010).

The River Stour rises at Stourhead in Wiltshire and is 97km long. The overall catchment area is approximately 1,240km^2 and has an associated population of around 400,000, mainly in Bournemouth and Christchurch. The river flows through a predominantly clay catchment in Blackmore Vale until Blandford Forum where it passes through a chalk escarpment before flowing towards and entering Christchurch Harbour. Important environmental sites in the catchment include two areas of natural beauty, three special areas of conservation, 13 sites of special scientific interest and two national nature reserves.

The Christchurch Harbour estuary is a natural harbour in Dorset UK and is fed by the River Stour and Hampshire Avon. The harbour is shallow, microtidal and due to the tidal harmonics in the English Channel has a double high water on each tide. The estuary flows into Christchurch Bay and the English Channel through a narrow channel known locally as The Run, which is situated between Mudeford Quay and Mudeford Spit. Christchurch Harbour is itself a site of special scientific interest and comprises a variety of habitats including, sand dune, wet meadows, saltmarsh and scrub as well as being a vital habitat for birds and estuarine associated organisms.

Four sample sites were chosen for this investigation (Figure 1.3), one at Knapp Mill (latitude = 50.748051; Longitude = -1.779946) on the Hampshire Avon, one at Throop (latitude = 50.763842; Longitude = -1.842014) on the River Stour, one at Iford Bridge (latitude = 50.740970; Longitude = -1.806441) on the River Stour and one at the mouth of Christchurch Harbour at the Run at Mudeford (latitude = 50.723573; Longitude = -1.742197).

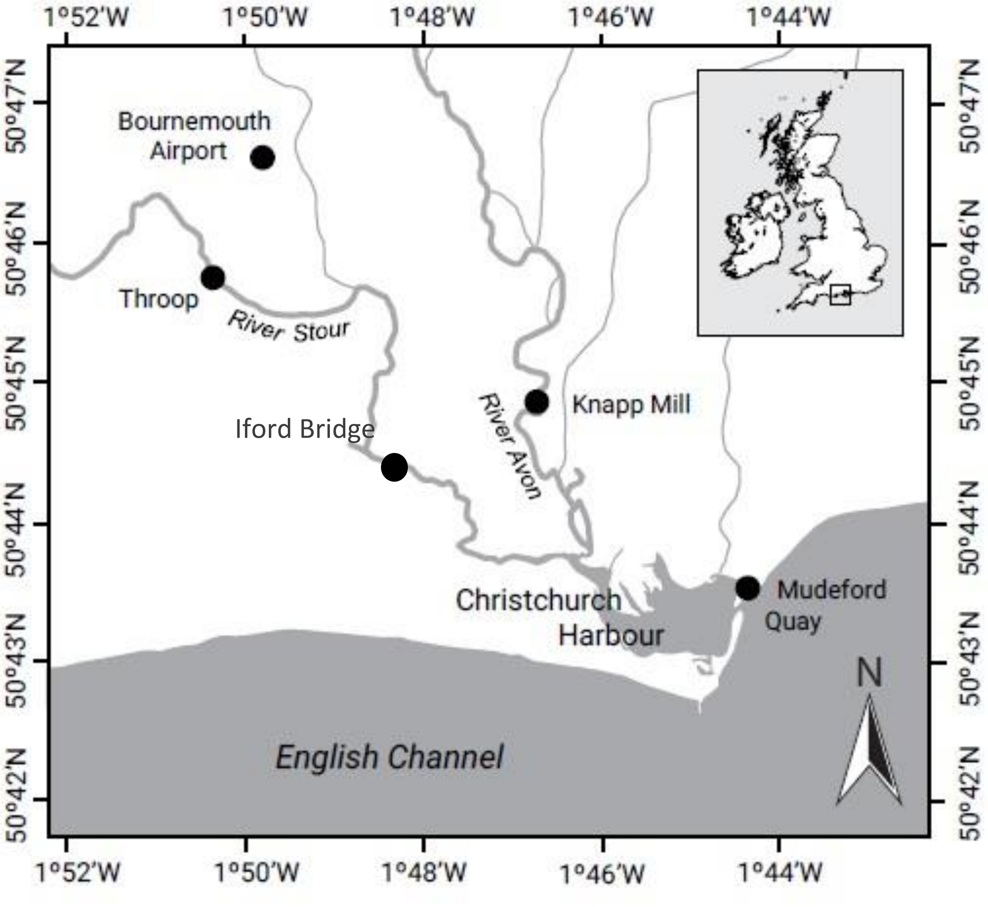


Figure 1.3: Location of the sample sites in relation to the UK.

1.6 NERC Macronutrients consortium

This research project was part of a consortium grant funded by the Natural Environmental Research Council (NERC) Macronutrients Cycles programme (MCP). The MCP had an overall budget of £9.5 million and ran from 2011-2015. The aim of the research programme was to quantify the scales (magnitude and spatial/temporal variation) of N and P fluxes and nature of transformations through the catchment under a changing climate and perturbed C cycle. In this description ‘'the catchment' is defined as covering exchange between the airshed and land surface through to the freshwater-estuarine boundary. This overall goal was to be achieved through four objectives.

1. To evaluate the nature and scale of macronutrient (N, C) exchange between the airshed and terrestrial system and consequences for losses (N, P, C) to freshwater and atmosphere systems (atmosphere-terrestrial-freshwater feedback system).
2. To determine the role and spatial and temporal variation of macronutrients (N, P, C) on key limiting processes and ecosystem functions (i.e. decomposition, productivity) and consequent export at the catchment scale (terrestrial-freshwater systems).
3. To advance understanding of the co-limitation of N/P for eutrophication control along the entire freshwater system to the estuarine boundary (freshwater system).
4. To determine the implications of nutrient enrichment on the fate and effects of other non-nutrient contaminants, including impacts on human health (i.e. pathogens, ozone) and biodiversity.

(nerc.ac.uk)

Four consortia grants and two proof-of-concept grants were awarded in order to fulfil these objectives.

This project formed part of the Christchurch Harbour Macronutrients Consortium (CHMC). The CHMC was designed to investigate the impact of stochastic storm events on the transport and biogeochemical cycling of macronutrients in two temperate rivers, the Hampshire Avon and the Stour, as well as in their shared estuary, Christchurch Harbour (UK). The overall aim was to ‘quantify annual fluxes, cycles and transformations of macronutrients in the Christchurch Harbour estuary, so as to better understand their responses to temporal patterns of change that have varying degrees of predictability.’ The focus of the research described in this thesis which formed one component of the CHMC consortium project was to investigate the seasonal changes in dissolved organic phosphorus in the lower reaches of the two rivers and Christchurch Harbour and to assess the relative importance of fluxes and transformations of DOP in relation to other factions of dissolved phosphorus in the river/estuary system. .

1.7 Thesis overview

1.7.1 Research questions

There are long-term datasets available of inorganic nutrient concentrations in most UK river systems and some estuaries from routine sampling undertaken by organisations such as the Environment Agency; however, the frequency of the sampling is rarely on more than a monthly basis. Additionally dissolved organic forms of N and P are not routinely measured by the Environment Agency or water companies and very few high frequency records of DOP and DON exist in UK rivers and estuaries. The overall goal of this research therefore was to investigate how the concentration of both dissolved inorganic and organic forms of P vary seasonally and with extreme changes in river flow in the lower reaches of the Hampshire Avon and Stour rivers and the receiving waters of the Christchurch Harbour estuary.

The following questions were addressed during the research:

- Can dissolved organic phosphorus (DOP) concentration be derived using a combination of inductively coupled plasma mass spectrometry (ICP-MS) to analyse total dissolved phosphorus (TDP) and a chemical assay to analyse (SRP)?
- How does DOP vary in a river and estuary system over the timescale of a year under varying conditions of river flow?
- How do inorganic nutrients (N, P and Si) behave in a river and estuary system over the timescale of a year?

1.7.2 Aims and Objectives of the thesis

The overall aim of the research presented in this thesis was to investigate the DOP dynamics in the lower reaches of two rivers (the Hampshire Avon and Dorset Stour) and the receiving waters of an estuary (Christchurch Harbour) over an annual period at high (i.e. weekly) sampling frequency.

The main objectives of this chapter are as follows:

Chapter 2: Inorganic nutrient dynamics in a river and estuary system.

In this chapter the data set of routine measurements made during the weekly sampling is reported with the following objectives.

- To monitor the annual changes in inorganic nutrient concentrations and fluxes using well established techniques.
- To monitor the response of inorganic nutrient concentrations to abnormally high river flow.
- To relate inorganic nutrient concentrations and fluxes to environmental factors such as flow rate and conductivity/salinity.

- To relate the inorganic nutrient concentrations to historic nutrient data to identify long-term trends or abnormal concentrations.

Chapter 3: Optimization of the determination of dissolved organic phosphorus in environmental water samples using a combination of ICP-MS and spectrophotometric colourimetric analysis.

The overall aim of this chapter was to develop a method of DOP analysis using a combination of ICP-MS to analyse TDP and a colourimetric assay involving molybdenum blue to analyse SRP (Murphy and Riley, 1962).

The objectives of this chapter are as follows:

- To first optimise the established technique of analysing SRP in both river and estuarine water samples.
- To use a range or organic phosphorus standards and a certified reference material to determine the effectiveness of ICP-MS as a technique to analyse TDP.
- To test the reproducibly of measurements of DOP concentration in collected river and estuary water samples from the difference between TDP concentration (determined by ICPMS) and SRP concentration (determined by colorimetric assay).

Chapter 4: Dissolved organic phosphorus in the Christchurch harbour system.

The main objectives of this chapter are as follows:

- To use the novel technique described in Chapter 3 to analyse a large number of stored water samples for TDP, SRP and DOP concentration.
- To monitor the response of these phosphorus fractions concentrations to abnormally high river flow.

- To relate phosphorus concentrations and fluxes to environmental factors such as flow rate and conductivity/salinity.
- To investigate the effects of estuarine mixing on phosphorus concentrations and identify any conservative/non-conservative behaviour.
- To describe phosphorus dynamics within Christchurch Harbour estuary having undertaken multiple transects of the estuary in the summer of 2014.

Chapter 2: Inorganic Nutrients in the Christchurch Harbour System

2.1 Introduction

Environmental changes of anthropogenic origin such as increases in agriculture, industry and sewage discharge have greatly increased the amount of nutrients in surface water since the industrial revolution (Soetaert et al., 2006; Heathwaite 2010). Excess nutrients can cause algal growth and increases the likelihood of large algal blooms (Dippner, 1998), causing numerous problems such as depletion of oxygen (Foley et al., 2012), changes to secondary production (Nielsen and Richardson 1996) as well as potentially altering phytoplankton species composition (Philippart et al., 2000).

Nitrogen is a non-toxic, non-flammable, colourless and odourless gas and makes up around 78% of the air on Earth. Nitrate (NO_3^-) is the most abundant stable inorganic species of nitrogen in well-oxygenated waters (Chester, 2009) and originates mainly from soil leaching, terrestrial run-off and point source inputs from anthropogenic sources such as industry or sewage treatment effluent. Nitrogen is utilised by a wide range of organisms as it is a major component of amino acids and numerous studies have indicated that nitrates can be a limiting nutrient in both oceanic (Moore et al., 2013) and coastal systems (Howarth & Moreno, 2006).

Phosphorus is a component of cell membranes and as a component of DNA, RNA and ATP. Phosphorus is present in rivers and estuaries in dissolved, particulate and colloidal phases as well as having significant organic and inorganic forms (Statham, 2012). The most common inorganic form of phosphorus is phosphate (PO_4^{3-}). The sources of phosphate into rivers include weathering of rocks, such as apatite, terrestrial run off and point source inputs from sewage treatment works and septic tank effluent. Phosphate is also often the limiting nutrient to plant growth in freshwater lakes, reservoirs, streams, and in the headwaters of estuarine systems (Correll, 1999; Elser et al., 2007).

Dissolved silicon is present in rivers almost exclusively as silicic acid (H_4SiO_4), and is derived from the weathering of silica and aluminosilica minerals (Chester, 2009). Unlike nitrate and phosphate, silica inputs are largely independent of anthropogenic sources. Silicates are utilised by diatoms and other siliceous organisms such as radiolarians and silicoflagellates, which are strictly dependent on silica to build their biomineralized cell walls. Marine diatoms precipitate 240×10^{12} mol Si per year, making them the major sink in the global Si cycle (Bondoc et al., 2016).

The main source of dissolved silicon to river water and estuaries is from weathering of rock minerals by naturally acidic rainwater (Drever, 1997). Silica is vital for the building rigid parts of the cellular structure of many phytoplankton including diatoms and radiolaria. The main ways in which humans affect silica concentrations in river and estuary systems is by modifications to catchment hydrology including the building of dams, and diverting waters for irrigation (Ragueneau et al., 2006). In numerous rivers, as opposed to N and P, silica fluxes to estuaries are decreasing over time, which may be related to numerous factors including increased growth and burial of diatomaceous algae associated with N and P enrichment and longer water residence times in reservoirs behind dams (Seitzinger et al., 2010).

The aim of this chapter was to investigate the inorganic nutrient dynamics and physiochemical structure of the River Avon and Stour at the gauging stations of Knapp Mill on the River Avon and Throop on the River Stour, in addition to an extra site on the River Stour, 3.6km downstream from Throop at Iford Bridge, and at Mudeford Quay at the mouth of the Christchurch Harbour estuary (Figure 1.3). Nutrient data was collected from all four sites on a weekly basis from April 2013 to April 2014 and this chapter includes a discussion of the processes influencing the changes in concentration and fluxes of these nutrients with respect to physiochemical parameters and data collected at the same sites from the Environment Agency.

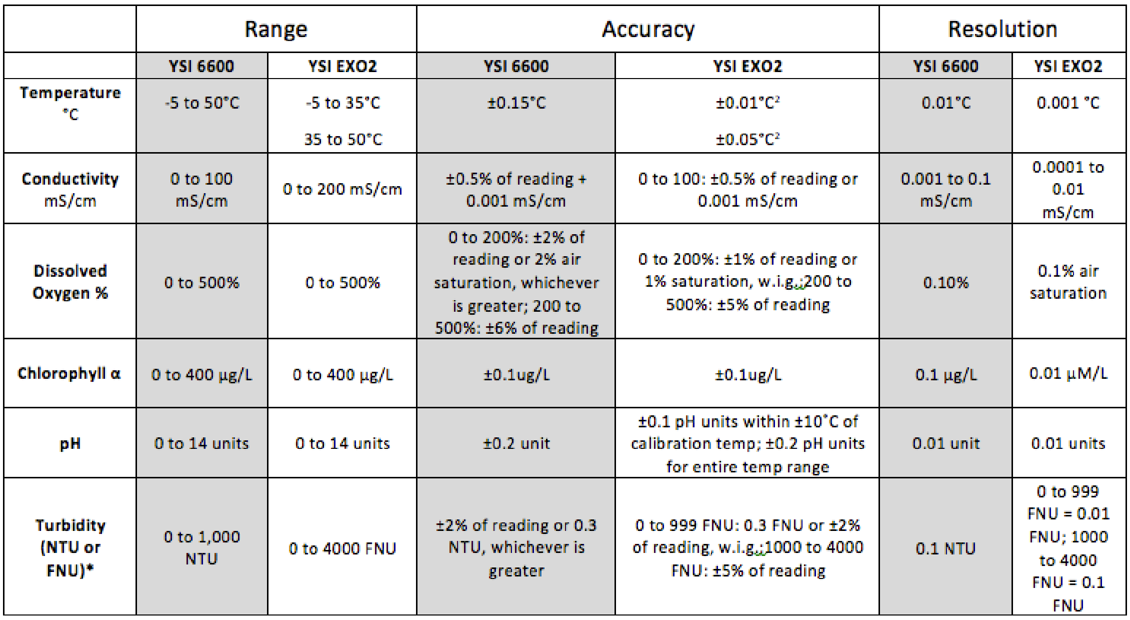
In addition to this, there was an exceptional period of prolonged high river flow in early 2014 and this chapter describes changes to inorganic nutrient concentrations and fluxes at a weekly resolution at four sites on two rivers and an estuary. Due to the high volume of water that can be transported down a river during a flood event, the transport of nutrients during this period could be significant and the high-resolution data gathered in this investigation allows nutrient dynamics during this period to be identified.

2.2 Methods

2.2.1 Sample collection

Water samples were collected from four sites, at Throop and Iford on the River Stour, at Knapp Mill on the River Avon and at the mouth of Christchurch Harbour at Mudeford Quay (Figure 1.3). At each site, a water sample of about 1 litre was collected from the surface using a pre-rinsed bucket and then decanted into pre-rinsed, 1L wide-mouth, polyphenylene ether (PPE) bottles. These were then transferred in a cool box back to the laboratory. Samples were collected on a weekly basis from 25/4/13 until 7/5/14, and then on a fortnightly basis from 7/5/14 to 13/8/14 at Knapp Mill, Throop and Mudeford Quay. Samples from an additional site at Iford on the River Stour 3.6km downstream from Throop were collected on a weekly basis from 6/6/13 to 8/4/14. At each site, a multiparameter sonde was deployed, and measurements of the temperature (^{o}C), conductivity (mS) or salinity (PSU), chlorophyll a (µg/L), dissolved oxygen (mg/L), oxygen saturation (%), turbidity (NTU (Nephlometric Turbidity Units) /FNU (Formazin Nephelometric Unit)), pH and total depth (m) were recorded. In total two sondes (detection limits are shown in table 2.1) were used, a YSI 6600 multi parameter sonde from 16/4/13 to 14/8/13 and a YSI EXO2 Multiparameter Sonde (Xylem analytics) for the rest of the sampling period. The change in sonde did not affect the majority of the parameters measured, as the units used in both instruments were the same. However, turbidity was measured in NTU on the YSI 6600 and in FNU on the YSI EXO2. FNU and NTU both are a measure of scattered light at 90 degrees but NTU is measured using a white light source and FNU is measured using a infrared light source.   For this reason, any figures containing turbidity data will only include data collected after 14/8/13.

Table 2.1: Detection limits for the multiparameter sondes used in this investigation



	Range		Accuracy		Resolution	
	YSI 6600	**YSI EXO2**	**YSI 6600**	**YSI EXO2**	**YSI 6600**	**YSI EXO2**
Temperature °C	-5 to 50°C	-5 to 35°C 35 to 50°C	±0.15°C	±0.01°C^2 ±0.05°C^2	0.01°C	0.001 °C
Conductivity mS/cm	0 to 100 mS/cm	0 to 200 mS/cm	±0.5% of reading + 0.001 mS/cm	0 to 100: ±0.5% of reading or 0.001 mS/cm	0.001 to 0.1 mS/cm	0.0001 to 0.01 mS/cm
Dissolved Oxygen %	0 to 500%	0 to 500%	0 to 200%: ±2% of reading or 2% air saturation, whichever is greater; 200 to 500%: ±6% of reading	0 to 200%: ±1% of reading or 1% saturation, w.i.g.;200 to 500%: ±5% of reading	0.10%	0.1% air saturation
Chlorophyll α	0 to 400 µg/L	0 to 400 µg/L	±0.1ug/L	±0.1ug/L	0.1 µg/L	0.01 µM/L
pH	0 to 14 units	0 to 14 units	±0.2 unit	±0.1 pH units within ±10˚C of calibration temp; ±0.2 pH units for entire temp range	0.01 unit	0.01 units
Turbidity (NTU or FNU)*	0 to 1,000 NTU	0 to 4000 FNU	±2% of reading or 0.3 NTU, whichever is greater	0 to 999 FNU: 0.3 FNU or ±2% of reading, w.i.g.;1000 to 4000 FNU: ±5% of reading	0.1 NTU	0 to 999 FNU = 0.01 FNU; 1000 to 4000 FNU = 0.1 FNU

*As two sondes were used over the course of this investigation, turbidity was measured in Nephelometric Turbidity Units (NTU) on the YSI 6600 and in Formazin Nephelometric Units (FNU) on the YSI EXO2.

2.2.2 Filtration

On return to the laboratory water samples were filtered on the same day as collected to minimise any chemical or biological changes to the sample. A sample of 65ml was immediately passed through a pre-combusted Whatman GF/F glass microfiber filter (retains particles down

0.7µm) and 20 ml added to a clean plastic vial to which 5µl of a 4g L^{-1} solution of mercuric chloride was added which can stabilise the phosphate concentration for at least 2 years (Kattner, 1999).

2.2.3 Analysis

Inorganic analyses were performed at the University of Portsmouth using a Seal Analytical QuAAtro autoanalyser. Channels were set up for total oxidisable N (nitrate-N plus nitrite-N), nitrite, silica and soluble reactive phosphorus (SRP) based on the colourimetric techniques of Henriksen and Selmer-Olsen (1970), Armstrong et al., (1967) and Murphy and Riley (1962) respectively. Total oxidisable N analyses were based on the hydrazine–copper reduction method which produces a reddish purple azo dye measured colourimetrically at 540 nm. Phosphate was also determined colourimetrically following reaction with heptamolybdate and potassium antimony (III) oxide tartrate in acidic solution followed by reduction with ascorbic acid, producing a blue colour, measured colourimetrically at 880 nm. Silicate was analysed by adding an acidic solution of ammonium molybdate to a sample to produce silicomolybdic acid, which was then reduced to silicomolybdous acid (a blue compound) following the addition of stannous chloride. Tartaric acid was added to impede PO_4 colour interference. Analyses were performed by various members of the Christchurch Harbour Macronutrients Consortium, with permanent members of staff at Portsmouth University responsible for the majority of the analysis. Detection limits of nutrients were analysed as three times the standard deviation of measurements of 5 Milli-Q ultrapure water samples. Detection limits of nitrate, phosphate, and silicate were 0.06, 0.01, and 0.05 µmol L^{-1}, respectively (Couceiro et al., 2013).

2.3 Results

2.3.1 Temperature

Temperature (^{o}C) at the 4 sites showed a clear seasonal signal over the sampling period. The temperature profiles at the 4 sites were similar throughout the sampling period (Figures 2.1, 2.2, 2.3 and 2.4), with the exception of Mudeford Quay from 6/8/13 to 3/9/13, where higher temperatures were recorded than at the other sites. The temperatures at the four sites peaked in July 2013 then decreased slowly to a minimum in late November 2013. There was then a slight increase in temperature from December 2013-January 2014 before the temperature began to rise towards June 2014. The highest and lowest temperatures respectively at Knapp Mill were 22.9°C on 23/7/13 and 5.7°C on 23/1/14, 22.8°C on 10/7/13 and 6.2°C on 13/2/14 at Throop, 22.3°C on 23/7/13 and 6.2°C on 13/12/13 at Iford and 23.0°C on 10/7/13 and 6.2°C on 23/1/14 at Mudeford (Table 2.2).

2.3.2 Conductivity/Salinity

The highest and lowest recorded conductivities respectively at Knapp Mill were 0.58mS/cm on 10/7/13 and 0.27mS/cm on 7/1/14, 0.59mS/cm on 23/7/13 and 0.28mS/cm on 7/1/14 at Throop, 0.58mS/cm on 10/7/13 and 0.27mS/cm on 7/1/14 at Iford and the salinity varied from 33.9 on 23/7/13 to 0.3 on 7/1/14 at Mudeford (Table 2.2; Figures 2.1, 2.2, 2.3 and 2.4). The conductivity at the three riverine sites showed similar seasonal patterns with higher conductivities recorded during the summer and lower conductivities recorded when the river flow was high.

2.3.3 Dissolved Oxygen Saturation

The highest and lowest dissolved oxygen saturation (DO %) respectively at Knapp Mill were 143.8% on 6/6/13 and 81.7% on 24/10/13, 143.0% on 10/5/13 and 77.5% on 1/10/13 at Throop, 118.9% on 6/6/13 and 75.7% on 1/10/13 at Iford and 150.5% on 10/7/13 and 78.7% on 24/10/13 at Mudeford (Table 2.2). DO % varied both weekly and seasonally at all four sites and there were differences in DO % between the sample sites. The DO % was highest from the period between April 2013 and October 2013.

At Knapp Mill and Mudeford Quay, the DO% was variable from 3/5/13 to 11/9/13 from 87.3% to 150.5%. During this period there was a reduction in dissolved oxygen saturation to approximately 90% from 14/6/13 to 28/6/13, coinciding with an increase in chlorophyll a concentration. During this variable period, the DO % at both Knapp Mill and Mudeford was similar. However, the DO% at Throop and Iford on the River Stour was lower on average and less variable than at Knapp Mill or Mudeford during this period. The DO% at all four sites was comparable between November 2013 to January 2014 at approximately 90% saturation (Figures 2.2, 2.3, 2.4 and 2.5).

2.3.4 Chlorophyll a concentration

The highest and lowest recorded chlorophyll a concentrations respectively at Knapp Mill were 9.0µg/L on 6/2/14 and 0.2µg/L on 9/12/13, 16.3µg/L on 14/6/13 and 0.4µg/L on 9/12/13 at Throop, 20.2µg/L on 14/6/13 and 0.5µg/L on 2/12/14 at Iford and 11.3µg/L on 3/5/13 and 0.7µg/L on 9/12/13 at Mudeford (Table 2.2).

In June 2013 at the three riverine sites there was a large peak in chlorophyll a concentration followed by a decrease until October 2013. There are then two more peaks in chlorophyll a concentration associated with increases in river flow in October 2013 and early 2014. The chlorophyll a concentration at Mudeford was higher than the chlorophyll a concentrations at both of the river sites from May 2013 to August 2013 (Table 2.2; Figures 2.1, 2.2, 2.3 and 2.4).

2.3.5 pH

The highest and lowest recorded pH at Knapp Mill were 9.6 on 6/6/13 and 7.7 on 16/12/13, 9.2 on 6/6/13 and 7.6 on 7/1/14 at Throop, 9.25 on 6/6/13 and 7.6 on 30/12/13 at Iford and 9.3 on 6/6/13 and 7.4 on 16/12/13 at Mudeford (Table 2.2; Figures 2.1, 2.2, 2.3 and 2.4).

2.3.6 Turbidity

The highest and lowest recorded turbidity at Knapp Mill were 0.8 FNU on 7/2/14 and 33.1 FNU on 27/7/13, 1.5 FNU on 27/8/13 and 105.2 FNU on 16/12/13 at Throop, 2.9 FNU on 27/3/14 and 83.1 FNU on 16/12/13 at Iford and 2.3FNU on 13/3/14 and 96.0 FNU on 4/11/13 at Mudeford (Table 2.2; Figures 2.1, 2.2, 2.3 and 2.4).

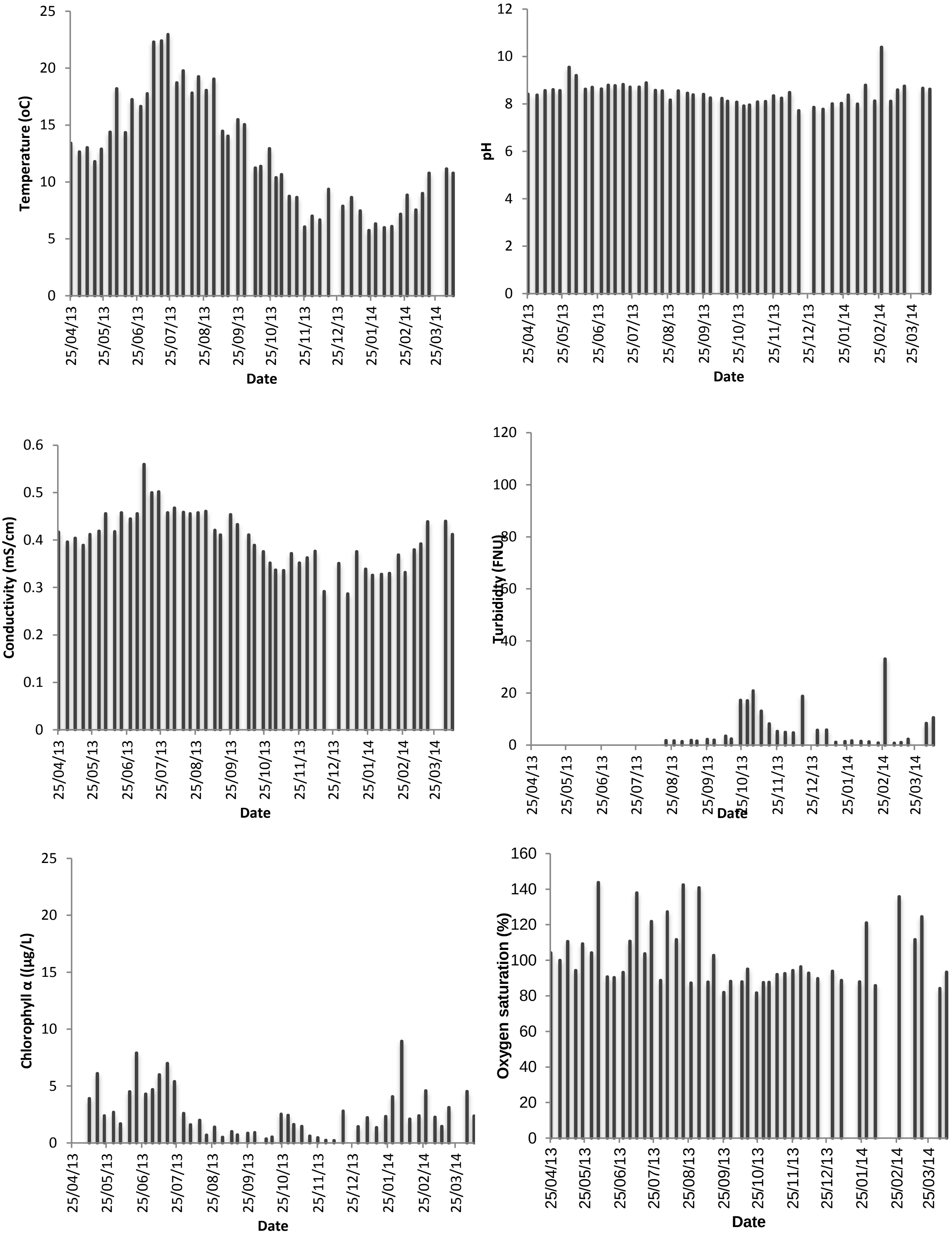


Figure 2.1: Sonde measurements taken on the same dates as the water samples at Knapp Mill on the River Avon

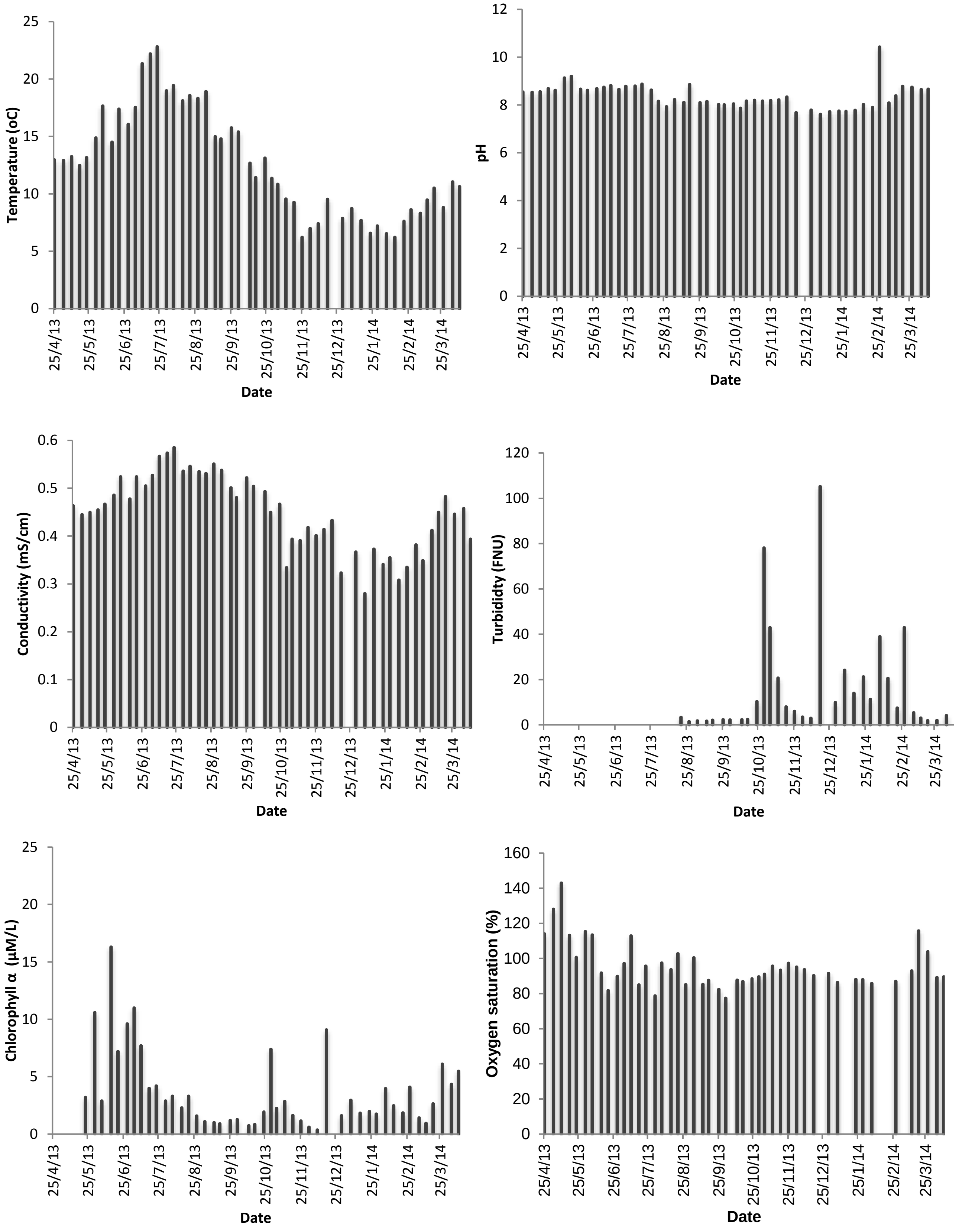


Figure 2.2: Sonde measurements taken on the same dates as the water samples at Throop on the River Stour

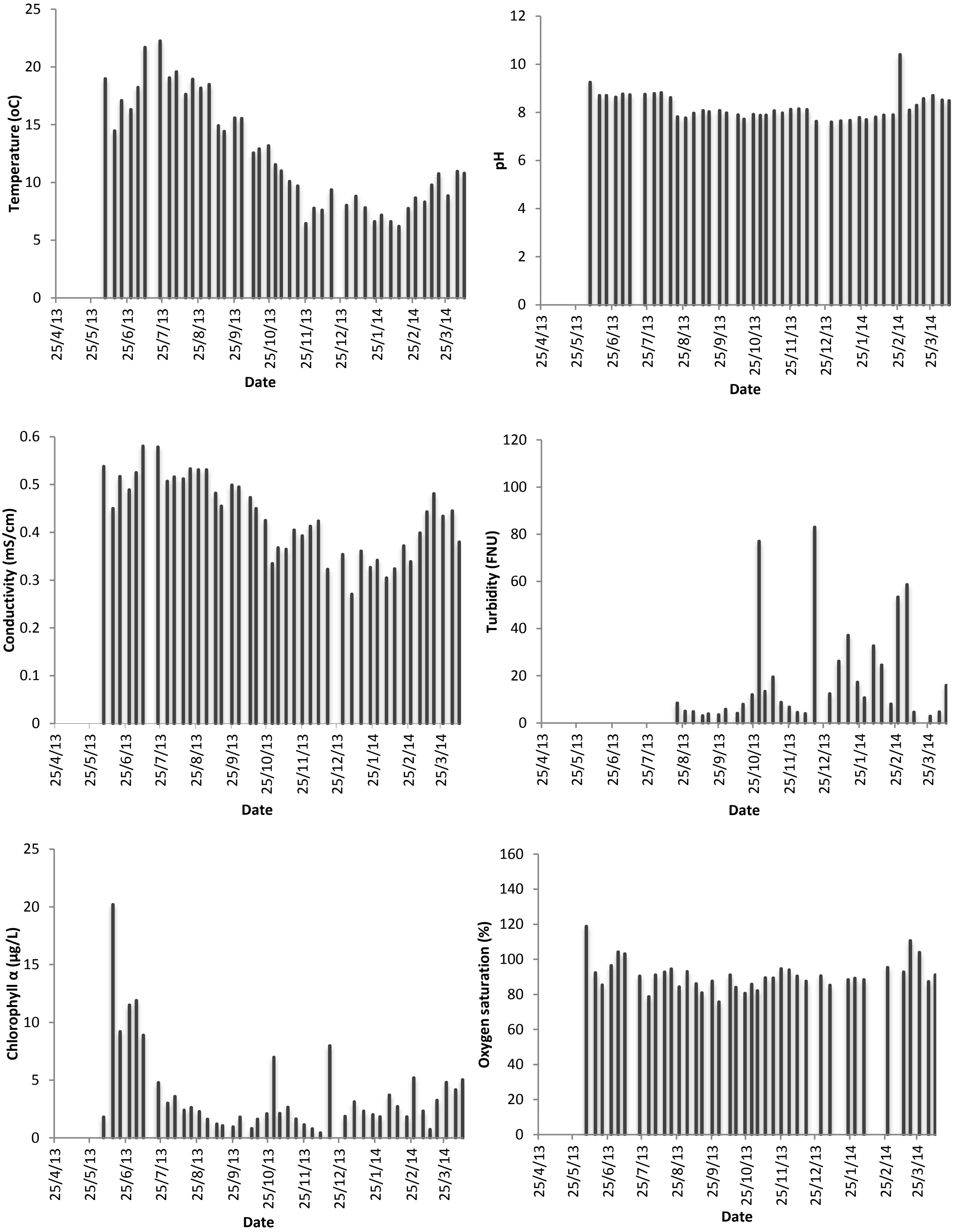


Figure 2.3: Sonde measurements taken on the same dates as the water samples at Iford Bridge on the River Stour

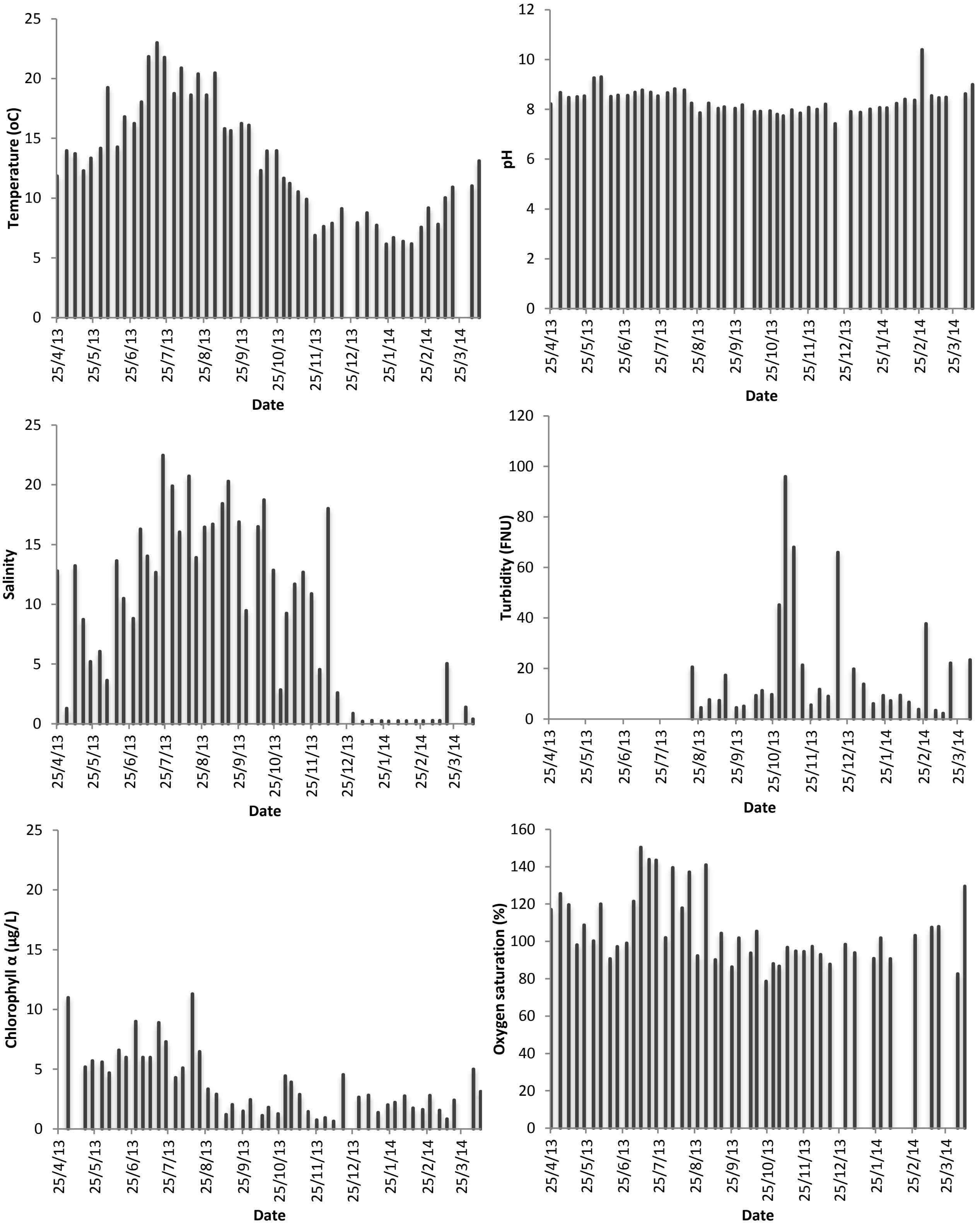


Figure 2.4: Sonde measurements taken on the same dates as the water samples at Mudeford Quay at Christchurch Harbour.

2.3.7 Mean daily flow rate

The mean daily flow rate at Knapp Mill on the River Avon and at Throop on the River Stour is measured by the Environment Agency. The mean flow rate at Knapp Mill was 25.5m^3/s with a maximum of 93.3m^3/s and a minimum of 5.8m^3/s. At Throop, the average flow rate was 18.6m^3/s with a maximum of 83.6m^3/s and a minimum of 2.4m^3/s (Table 2.2). The flow rate at Mudeford was calculated as the sum of the mean daily flow rate at Knapp Mill and the mean daily flow rate at Throop. At Iford, the flow rate from the gauging station at Throop is used. The flow rate at Knapp Mill and Throop decreased from 23.7m^3/s and 16.4m^3/s on 16/4/13 to approximately half this rate in September respectively. At both sites there is a peak in flow rate from the 16/10/13 to 18/11/13 before a large peak in flow rate from 16/12/13 to 13/3/14. Both of these peaks coincide with an increase in monthly rainfall in the area.

Table 2.2: Minimum, maximum, mean and standard deviation of the measurements taken from the YSI EXO 2 Sonde and river flow data from the environment agency for the duration of the sampling period.

		Knapp Mill	Throop	Iford	Mudeford Quay
Temperature (°C)	Max	22.96	22.82	22.25	23.00
	Min	5.74	6.22	6.21	6.16
	Mean	12.62	12.74	12.58	13.20
	Standard deviation	4.88	4.64	4.73	4.83
Conductivity (ms/cm)	Max	0.56	0.59	0.58	-
	Min	0.29	0.28	0.27	-
	Mean	0.40	0.45	0.43	-
	Standard deviation	0.06	0.08	0.08	-
Salinity	Max	-	-	-	22.48
	Min	-	-	-	0.21
	Mean	-	-	-	9.17
	Standard deviation	-	-	-	7.23
pH	Max	10.4	10.4	10.4	10.4
	Min	7.7	7.6	7.6	7.4
	Mean	8.5	8.4	8.2	8.4
	Standard deviation	0.5	0.5	0.5	0.5
Chlorophyll a (µg/L)	Max	9.0	16.3	20.2	11.3
	Min	0.2	0.4	0.5	0.7
	Mean	2.7	3.7	3.8	3.8
	Standard deviation	2.1	3.4	3.8	2.7
Oxygen saturation (%)	Max	143.8	143.0	118.9	150.5
	Min	81.7	77.5	75.7	78.7
	Mean	102.2	95.7	91.0	106.1
	Standard deviation	17.8	13.1	8.4	18.5
Turbidity (FNU)*	Max	33.1	105.2	83.2	
	Min	0.8	1.5	2.9	2.3
	Mean	6.4	15.8	18.3	18.6
	Standard deviation	7.6	23.5	21.4	21.7
Mean daily flow rate (m^3/s)**	Max	102.64	139.54	139.52	201.44
	Min	5.67	2.43	2.43	8.13
	Mean	28.64	22.32	22.32	50.23
	Standard deviation	29.27	28.91	28.91	55.73

*Turbidity data are only presented from 20/8/13 to 10/4/14 because the two sondes used in this investigation calculated the turbidity using different units.

**Data for mean daily flow rate are from the Environment Agency gauging stations at Knapp Mill and Throop. The flow rate shown for Iford is assumed to be the same as at Throop and the flow rate shown for Mudeford is defined as the sum of the flow rate measured at Knapp Mill and Throop

2.3.8 Nitrate concentration

The weekly values of nitrate concentration at Knapp Mill, Throop, Iford and Mudeford are presented in Figure 2.5 with accompanying river flow data. The average nitrate concentration at Knapp Mill was 391.6µM (58 samples), at Throop 508.9µM (57 samples) at Iford 545.6µM (44 samples) and at Mudeford it was 319.3µM (59 samples) (Table 2.3). Nitrate concentrations remained relatively constant during periods of high river flow (>50m^3/s) (Figure 2.5) with only a small reduction in the nitrate concentration during the period of high river flow in early 2014. The highest nitrate concentrations occurred in March 2014 at all three riverine sites, as the large rainfall event ended and the river flows decreased back to base levels. The average nitrate concentrations were higher at Iford and in addition to this, large spikes in nitrate concentration were evident at Iford, which were not identified at Throop e.g. 30/5/13, 14/6/13 and 10/10/13.

Table 2.3: Minimum, maximum, mean and standard deviation of the inorganic nutrients for the duration of the sampling period

		Knapp Mill	**Throop**	**Iford**	**Mudeford Quay**
Nitrate	Max (µM)	524.1	675.1	826.4	545.8
	Min (µM)	288.6	346.3	334.5	67.9
	Mean (µM)	391.6	508.9	545.6	319.3
	Standard deviation	58.5	67.6	110.3	114.8
Nitrite	Max (µM)	5.8	6.0	6.7	6.5
	Min (µM)	0.8	1.3	1.4	0.7
	Mean (µM)	3.0	3.5	3.4	2.6
	Standard deviation	1.3	1.2	1.1	1.2
Phosphate	Max (µM)	3.2	25.2	48.7	10.9
	Min (µM)	0.1	1.5	3.3	1.1
	Mean (µM)	1.7	10.2	17.3	5.3
	Standard deviation	0.8	6.4	12.4	2.9
Silicate	Max (µM)	240.1	216.3	190.7	188.8
	Min (µM)	4.5	5.7	47.5	14.6
	Mean (µM)	135.4	104.3	112.8	84.1
	Standard deviation	63.4	40.1	32.2	47.2

In addition to nitrate analysis, other forms of N can be bioavailable and play a major role in estuarine biogeochemistry. These alternate forms of N include Nitrite (Figure 2.6), Ammonium and organic nitrogen compounds. In this investigation, the proportion of total inorganic nitrogen (nitrate + nitrite) that was nitrite was consistently low, with a mean proportion at Knapp Mill of 0.8% at Throop of 0.7% at Iford of 0.6% and at Mudeford of 0.8%.

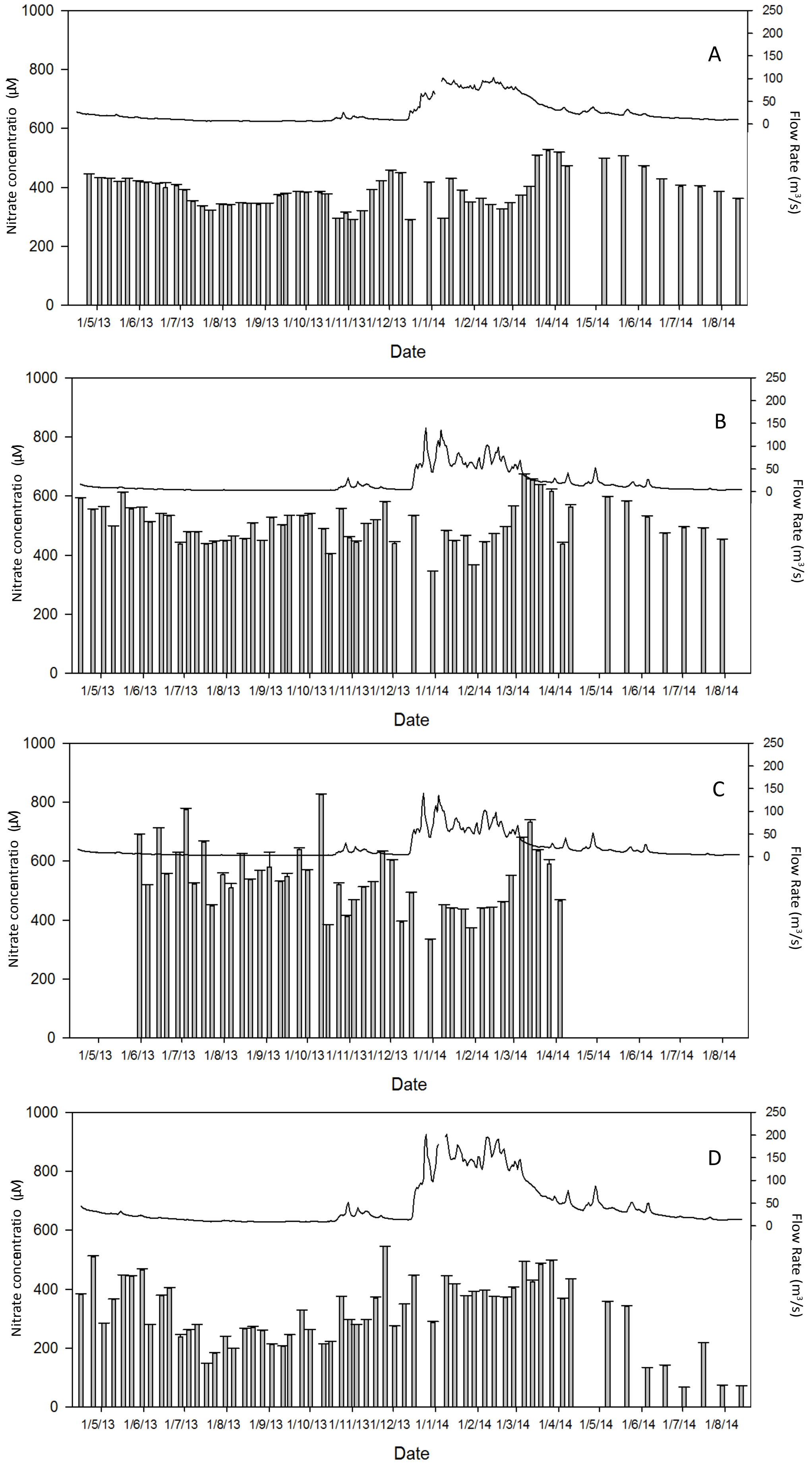


Figure 2.5: Nitrate concentrations and river flow rate at Knapp Mill (A), Throop (B), Iford (C) and Mudeford (D). Error bars show +- 1 sd from 3 analytical measurements

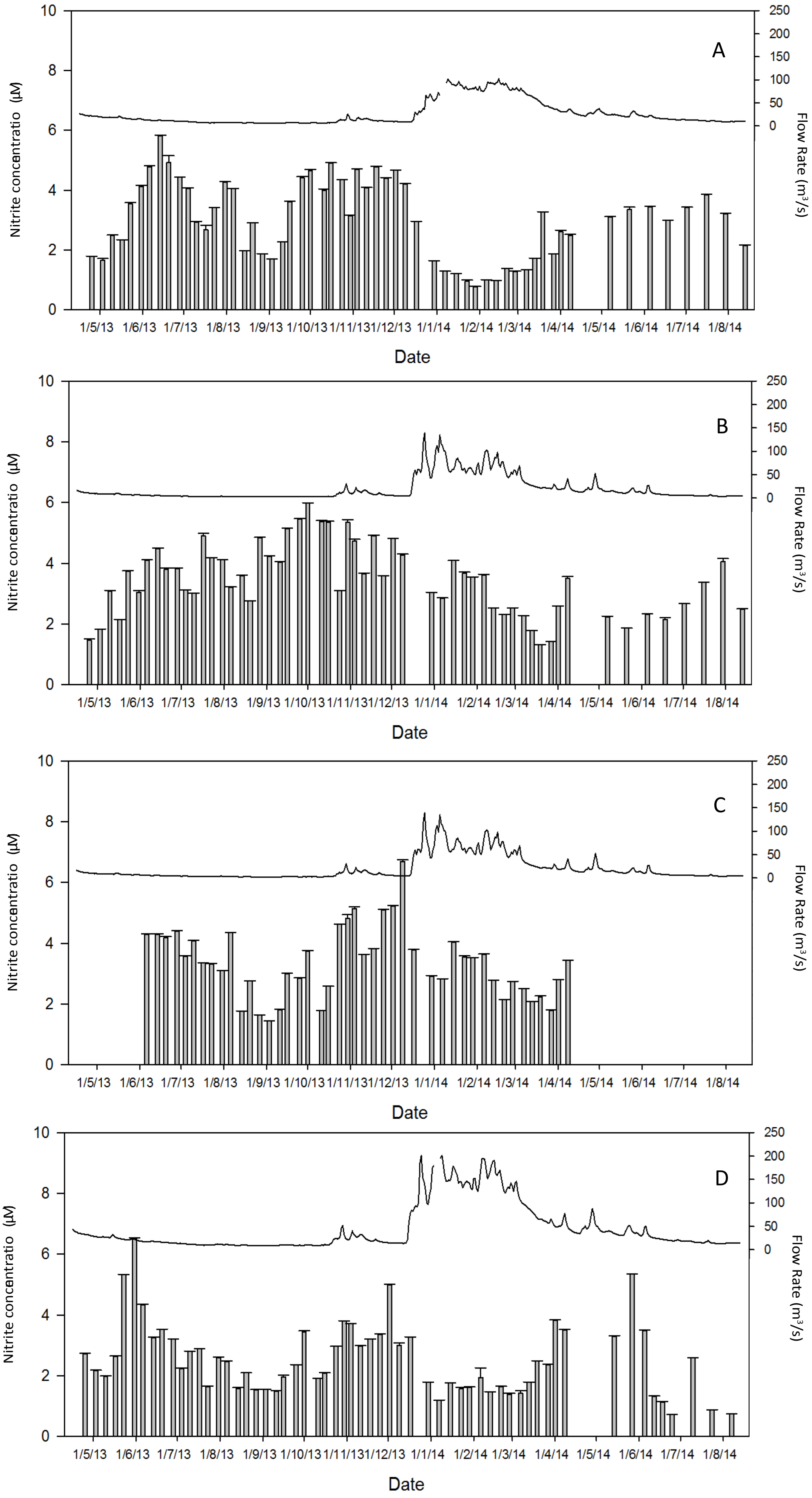


Figure 2.6: Nitrite concentrations and river flow rate at Knapp Mill (A), Throop (B), Iford (C) and Mudeford (D). Error bars show +- 1 sd from 3 analytical measurements

2.3.9 Phosphate concentration

The weekly measured values of phosphate concentration at Knapp Mill, Throop, Iford and Mudeford are presented in Figure 2.7 with accompanying river flow data. The average SRP concentration at Knapp Mill was 1.6µM (58 samples), at Throop it was 10.1µM (57 samples) at Iford it was 17.3µM (44 samples) and at Mudeford it was 5.3µM (59 samples) (Table 2.3). SRP concentrations at the three riverine sites showed a clear seasonal trend with the lowest SRP concentrations during the January to April period, followed by a steady increase from May to August. The highest SRP concentration at Knapp Mill was 3.28µM on 7/1/14, at Throop the highest concentration was 25.2µM on 25/9/13 at Iford the highest SRP concentration was 48.7µM on 16/10/13 and the highest SRP concentration at Mudeford Quay was 10.9µM on 23/9/13. At Mudeford Quay the SRP concentration was high (>10µM) from May 2013 to December 2013 with low concentrations associated with high river flow from December 2014 to April 2014.

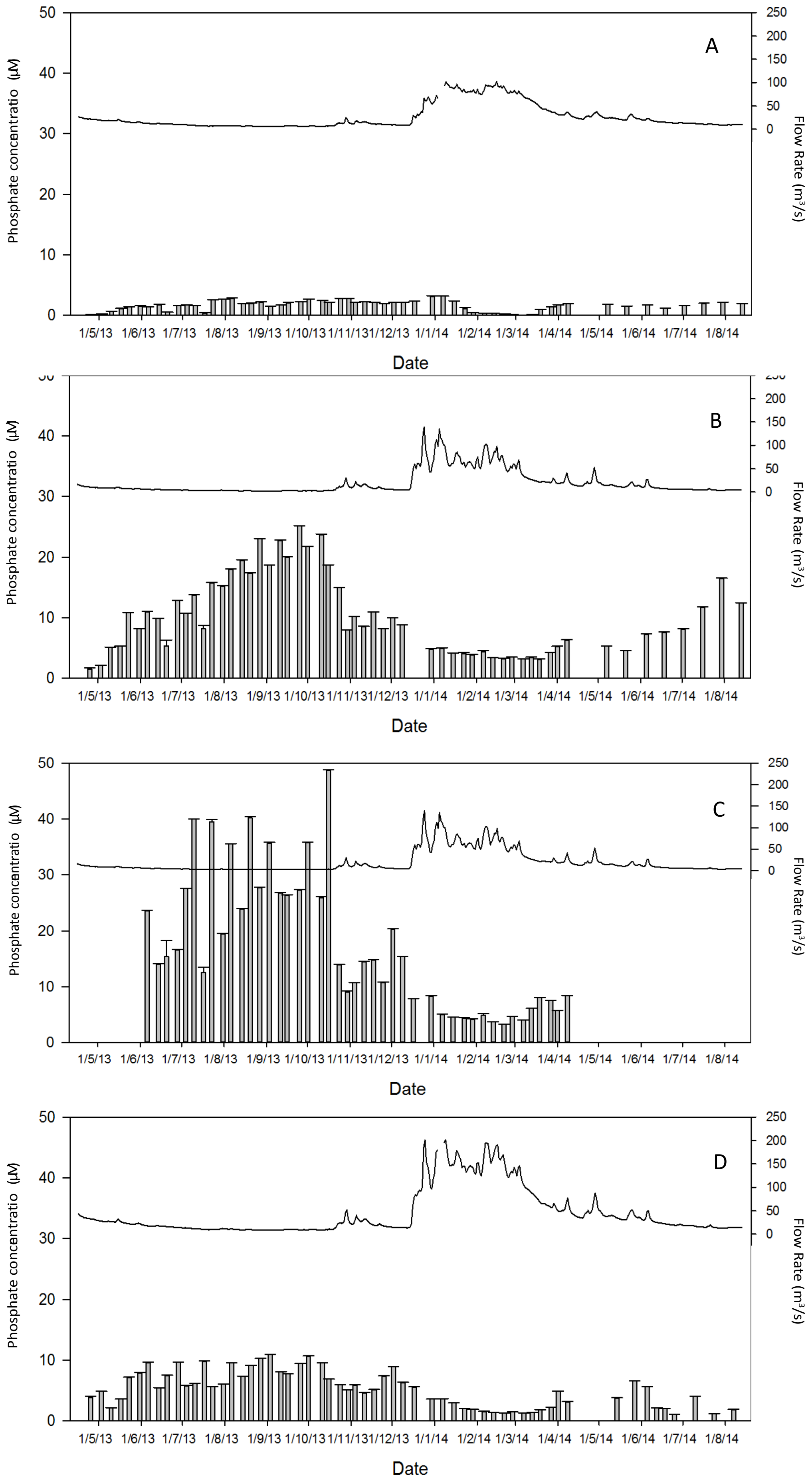


Figure 2.7: Phosphate concentrations and river flow rate at Knapp Mill (A), Throop (B), Iford (C) and Mudeford (D). Error bars show +- 1 sd after 3 analytical repeats.

2.3.10 Silica concentration

The weekly measurements of silica concentration made on samples collected from Knapp Mill, Throop, Iford and Mudeford are presented in 2.8, with accompanying river flow data. The average silica concentration at Knapp Mill was 135.4 (58 samples), at Throop it was 104.2µM (57 samples) at Iford it was 112.7µM (44 samples) and at Mudeford it was 84.0µM (59 samples) (Table 2.3). As with SRP and nitrate, the riverine sites showed a clear seasonal signal with regard to changes in silica concentration. The lowest silica concentrations detected from January to April 2014, followed by a steady increase from May to August. In contrast to nitrate and phosphate, silica concentrations were highest in the River Avon. There is a drop in silica in early 2014 at all sites, but it is less pronounced at Knapp Mill on the River Avon (Figure 2.8). However, the average silica concentration at Mudeford was lower than at Knapp Mill, Throop and Iford.

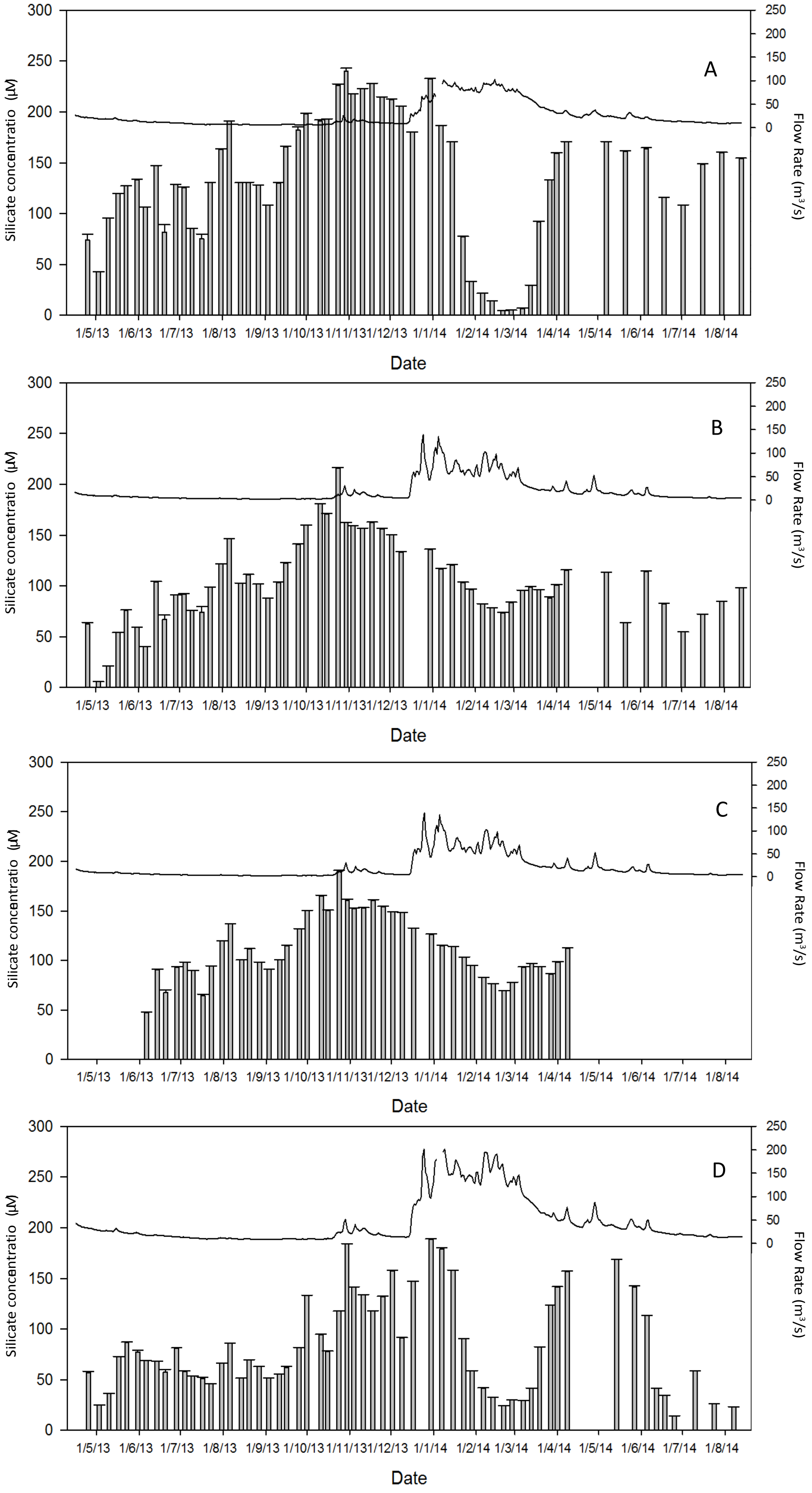


Figure 2.8: Silica concentrations and river flow rate at Knapp Mill (A), Throop (B), Iford (C) and Mudeford (D). Error bars show +- 1 sd after 3 analytical repeats.

2.3.11 Nutrient fluxes

Fluxes of nitrate, nitrite, silica and phosphate were calculated using the inorganic nutrient concentrations from the analyses at the University of Portsmouth and the daily mean river flow data from the Environment Agency gauging stations at Knapp Mill on the River Avon (Figure 2.9) and Throop on the River Stour (Figure 2.10). Fluxes were calculated by multiplying the measured concentration of each nutrient by the daily mean flow for the same day at that site (equations are shown below). For the estuary mouth at Mudeford (sampled at low tide) the daily mean flow for both rivers were summed together to give an estimate of the total daily mean flow which was then multiplied by the nutrient concentrations measured at Mudeford.

$$\text{Mean daily nutrient flux (M)} = \frac{mean\ daily\ flow\ rate\ (L) \times concentration\ of\ nutrient\ (\mu M)}{1000000}$$

$$\text{Mean daily nutrient flux } \left(\frac{Kg}{day}\right) = \frac{Mean\ daily\ nutrient\ flux\ (M) \times nutrient\ relative\ atomic\ mass}{1000}$$

The maximum flux of nitrate over the sampling period at Knapp Mill was 1 x 10^5 kg/day on 15/01/14 and 0.9 x 10^5 kg/day on 13/2/14 at Throop. The minimum flux of nitrate was 2111.9 kg/day on 7/8/14 at Knapp Mill and 940 kg/day also on 7/8/14 at Throop. The mean flux of nitrate was 2.4 x 10^4 kg/day at Knapp Mill and 2.3 x 10^4 kg/day at Throop. The nitrate fluxes were similar at Knapp Mill and Throop over the sampling period. The nitrate flux decreased slowly from May 2013 to August 2013. The flux of nitrate stayed at a low level until November 2013 where there was an increase in nitrate flux at both sites, however this peak was more pronounced at Throop. The flux returned to a low level in December 2013 before increasing dramatically with the large high flow event in early 2014. At Throop this increase in nitrate flux remained at a high level until mid March 2014 before decreasing rapidly whereas at Knapp Mill the nitrate flux peaked early in the high flow event and reduced slowly over the next 8 months before returning to a base level in August 2014. There was another peak in nitrate flux at Throop in late May 2014, which was not observed at Knapp Mill. Nitrate flux was positively correlated with flow rate at both riverine sites (Appendix 3).

The SRP flux at Knapp Mill was relatively steady throughout the year, remaining below 180 kg/day except for the major peak in early 2014 associated with high rainfall. The maximum flux of phosphate over the sampling period at Knapp Mill was 549.5 kg/day on 15/01/14 and 766.8 kg/day on 13/2/14 at Throop. The minimum flux of phosphate was 7.6 kg/day on 17/7/13 at Knapp Mill and 24.4 kg/day on 7/8/14 at Throop. The mean flux of phosphate was 69.0 kg/day at Knapp Mill and 264.9 kg/day at Throop. The phosphate flux was consistently higher at Throop than at Knapp Mill from May 2013 to October 2013. There was then a small period of increased river flow in late October 2013 coinciding with a large peak in phosphate flux at Throop. This peak is present at Knapp Mill but is considerably smaller. Phosphate flux is positively correlated with flow rate at Throop and Knapp Mill (Appendix 3). The phosphate flux at Throop was elevated for the period of high flow in early 2014. The elevated river flow during this time period also caused an increase in phosphate flux at Knapp Mill. However, at Knapp Mill, the phosphate flux returned to a lower level after 2 weeks.

The maximum flux of silica over the sampling period at Knapp Mill was 3.5 x 10^4 kg/day on 15/01/14 and 1.9 x 10^5 kg/day on 13/2/14 at Throop. The minimum flux of silica was 677.6 kg/day on 7/8/14 at Knapp Mill and 162.3 kg/day on 3/5/13 at Throop. The mean flux of silica was 5566.0 kg/day at Knapp Mill and 4578.7 kg/day at Throop. The silica flux at Knapp Mill is consistently higher than at Throop from May to October 2013. There is a rise in silica flux associated with an increase in flow rate in October 2013 at both sites and silica flux is positively correlated with flow rate at Throop and Mill (Appendix 3). The silica flux at Knapp Mill increases after extended rainfall in early 2014 before returning to a low level in March 2014. The silica flux at Knapp Mill increases again in March 2014. The silica flux at Throop also increases due to a high flow event in early 2014 but remains at a high level for an extended period of time. The silica flux decreases in late March 2014 before increasing again in May 2014.

The nitrite flux was a much lower percentage of the total nitrogen flux than nitrate at Knapp Mill (0.2%) and Throop (0.6%). The maximum flux of nitrite over the sampling period at Knapp Mill was 326 kg/day on 21/02/13 and 605.3 kg/day on 29/1/14 at Throop. The minimum flux of nitrite was 21.5 kg/day on 7/8/14 at Knapp Mill and 9.6 kg/day on 7/8/14 at Throop. The mean flux of nitrite was 128.6 kg/day at Knapp Mill and 138.5 kg/day at Throop. The nitrite fluxes at Throop were relatively steady from May 2013 to late October 2013. However at Knapp Mill there is an increase in nitrite flux from May 2013 to mid June 2013. The nitrite flux then decreases before another increase in nitrite flux in mid November 2013 associated with river flow increase. This increase is also present at Throop but is more pronounced. The flux levels at both sites return to low levels in December 2013. During the high flow event in early 2014 the nitrite flux at Throop increases dramatically and remains high until March 2014 where it decreases over the course of 5 weeks. An increase in nitrite flux is observed at Knapp Mill but the flux is lower than at Throop and is sustained at this level until April 2014 where the nitrite flux decreases before returning to a base level in July 2014.

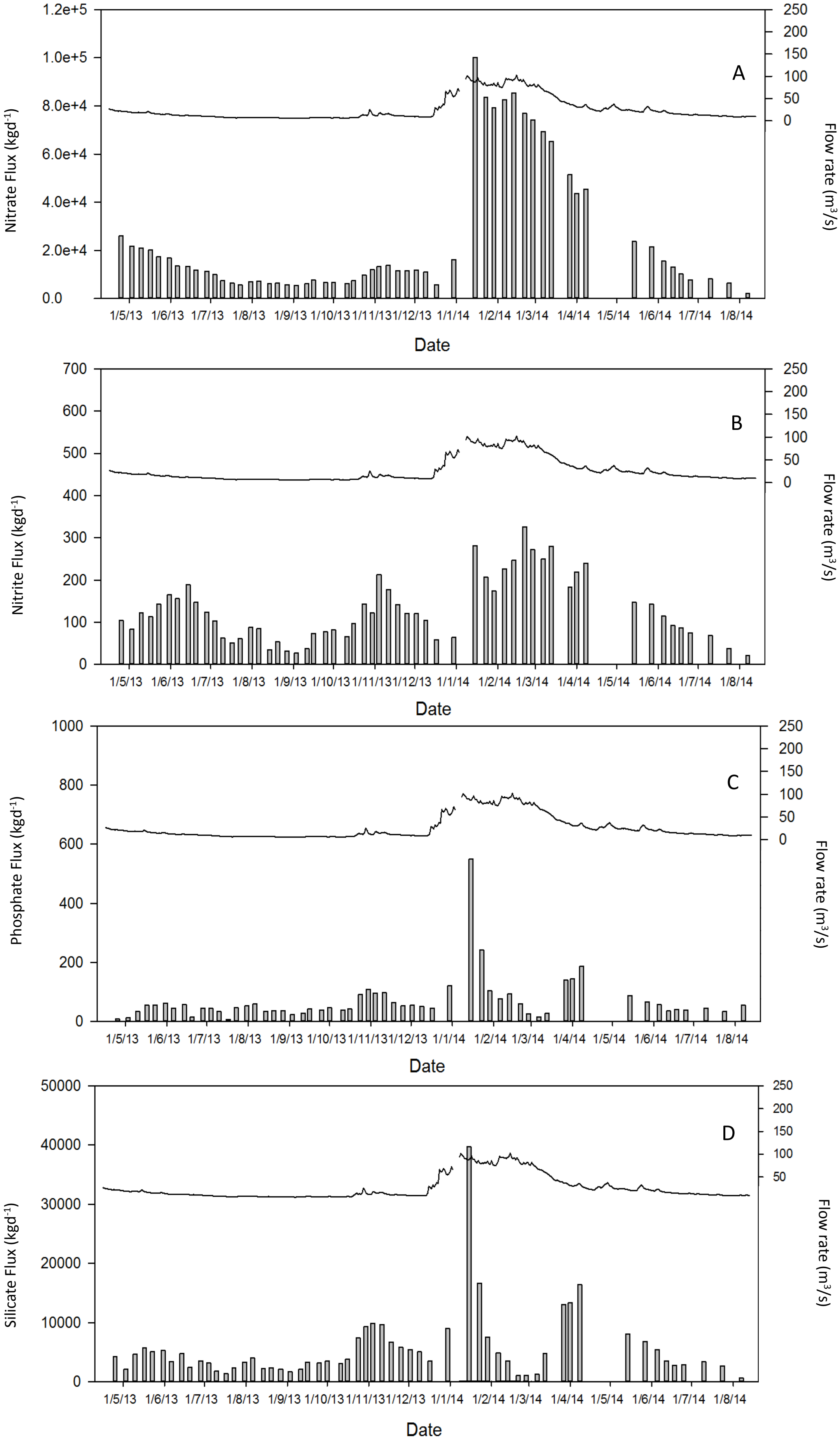


Figure 2.9: Flux of nitrate (A), nitrite (B), phosphate (C) and silica (D) at Knapp Mill on the River Avon for the duration of the sampling period.

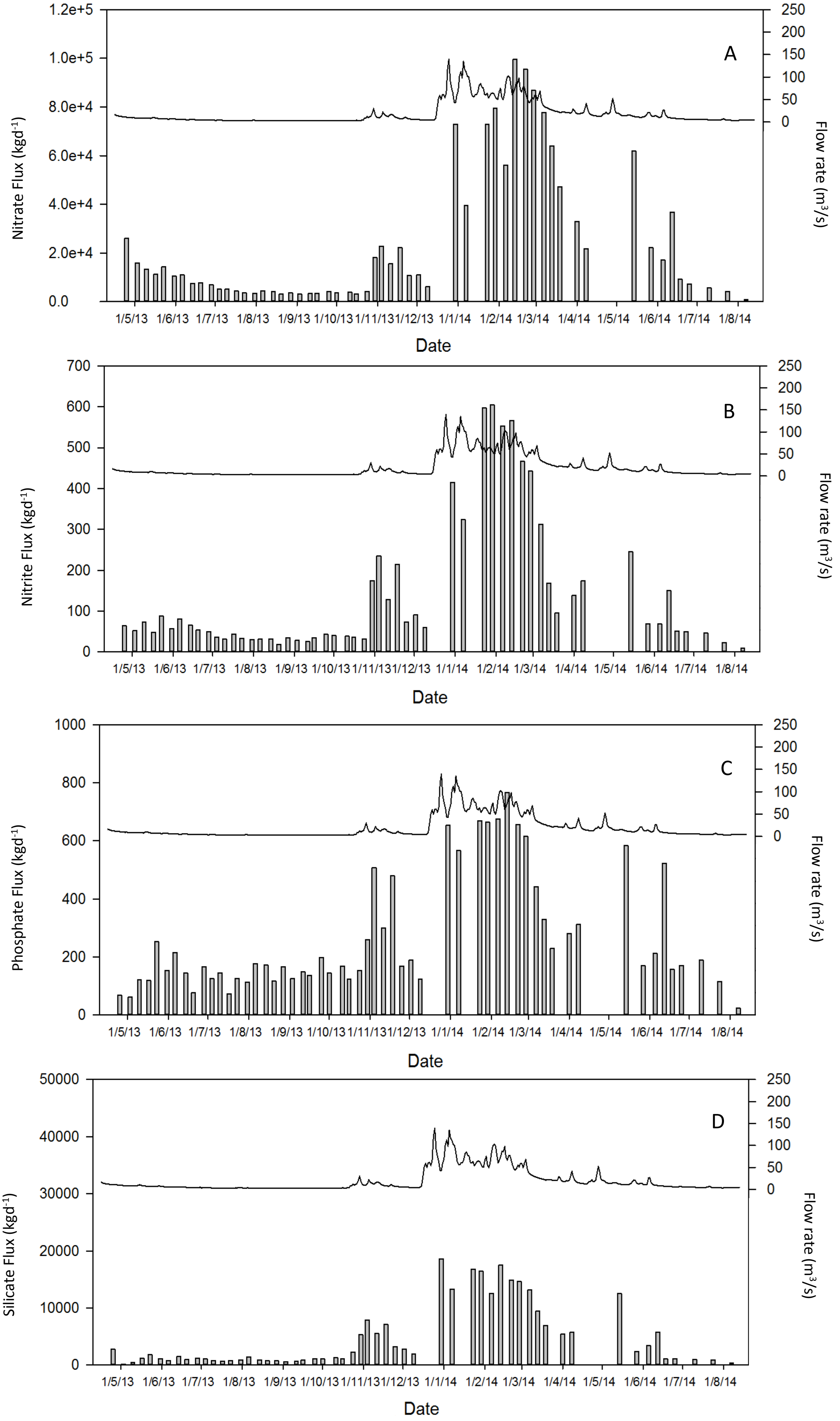


Figure 2.10: Flux of nitrate (A), nitrite (B), phosphate (C) and silica (D) at Throop on the River Stour for the duration of the sampling period.

2.3.12 Historic Nutrient data

The Environment Agency have gauging stations at Knapp Mill on the River Avon and Throop on the River Stour and have been monitoring the water quality of the rivers for a number of years. Figures 2.11 and 2.12 show the daily mean flow rate, phosphate concentration and nitrate concentration from 2000-2015. Nitrate and phosphate were sampled and analysed on approximately a monthly basis. Also shown are the nitrate and phosphate concentrations obtained during the current investigation (in red). River flow rate at both sites showed seasonal variation, with higher flows recorded during winter than in summer. The sampling period of this investigation included two periods of high river flow, in October 2013 and a larger one in early 2014. The high river flow period in early 2014 showed the highest river flows seen at Knapp Mill since January 2003 and also showed exceptionally high river flow at Throop (Figures 2.11 and 2.12).

Phosphate concentrations at Throop and Knapp Mill also show seasonal variation with high concentrations in spring reducing to autumn with low phosphate concentrations over winter. The phosphate concentration was higher and more variable at Throop than at Knapp Mill and there is an overall decreasing trend in phosphate concentration at Knapp Mill since 2000. Additionally, during the period in which this investigation took place the lowest phosphate concentrations at Knapp Mill were recorded since 2000.

Nitrate concentrations are higher on average at Throop than Knapp Mill and are more variable. A similar seasonal signal can be observed but it is less pronounced than with phosphate concentration. At Knapp Mill, the nitrate concentration in April 2014 was the highest recorded nitrate concentration post 2000.

For both phosphate and nitrate, the higher resolution of the sampling regime of this investigation (weekly compared to the Environment Agency's routine sampling (monthly) enables identification of short term changes in nutrient concentrations, especially with regard to high flow periods.

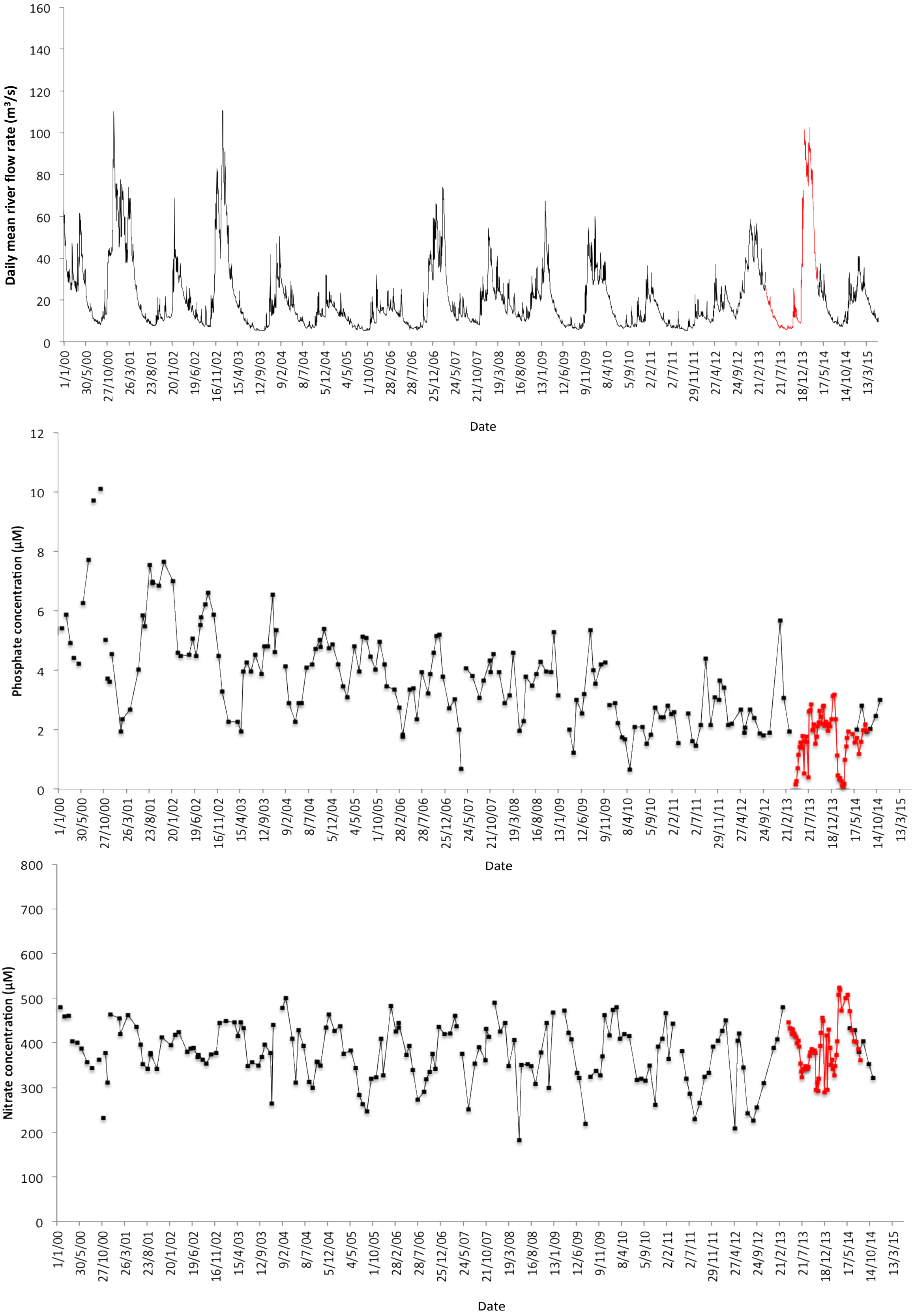


Figure 2.11: Mean daily flow rate, phosphate concentration and nitrate concentration at Knapp Mill on the river Avon from 1/1/00 to 1/10/15 from the Environmental Agency's routine sampling. For flow rate, the red points indicate the flow rate during the period of this investigation (April 2013-April 2014), for nitrate and phosphate, the red values indicate the concentrations derived from the inorganic analysis at the University of Portsmouth.

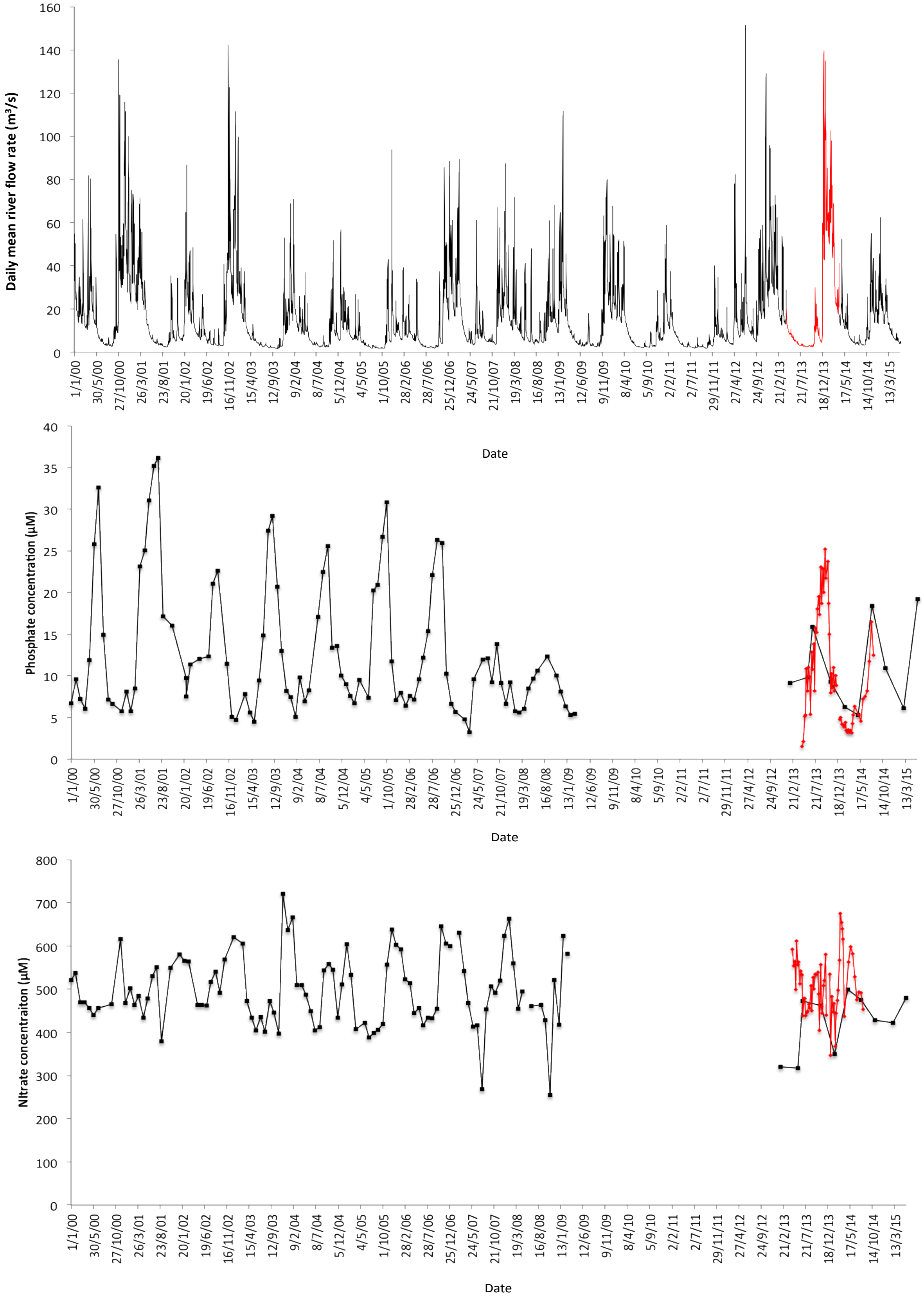


Figure 2.12: Mean daily flow rate, phosphate concentration and nitrate concentration at Throop on the river Stour from 1/1/00 to 1/10/15 from the Environmental Agency's routine sampling. For flow rate, the red points indicate the flow rate during the period of this investigation (April 2013-April 2014), for nitrate and phosphate, the red values indicate the concentrations derived from the inorganic analysis at the University of Portsmouth.

2.4 Discussion

2.4.1 Physiochemical parameters

Water temperature at the four sites showed expected seasonal variation with higher temperatures over summer and colder temperatures over winter. pH values were similar at the four sites but Knapp Mill had the highest mean pH over the sampling period, likely due to the underlying geology. The River Avon catchment system is mainly chalk based and is groundwater dominated with chalk aquifers. This may increase the pH due to the presence of calcium carbonate in the catchment system (Neal et al., 2002). Conversely, the river Stour has a more clay-based catchment. Conductivity is a measure of the concentration of ions in a water sample, the higher the ionic concentration of a water sample, the higher the conductivity. The conductivity at all 3 riverine sites was highest over summer and decreased into winter. This is due to high volumes of low-conductivity rainwater diluting the riverine water. During these times of high flow, the difference in conductivity between the River Avon and the River Stour was minimal. Conductivity at Knapp Mill on the River Avon was consistently lower than at the two sites on the River Stour. This may be due to differences in the bedrock of the two rivers; the River Avon catchment is high in chalk whereas the River Stour catchment is higher in clays. Clay soils tend to have higher conductivity because of the presence of materials that ionize when washed into the water (EPA, 2012).

Phytoplankton produce oxygen during hours of daylight, due to this, diurnal signals have been observed in dissolved oxygen saturation in previous studies (Neal et al., 2002). However, the weekly frequency of the sampling regime in this investigation was not high enough resolution to observe these effects. Nevertheless, variations in DO % can be observed in this investigation. The DO % at all four sites was comparable between November 2013 to January 2014, at approximately 90% saturation. During this time, Iford was the lowest in DO %, most likely due to respiration activity caused by sewage effluent from Holdenhurst sewage works (Daniel et al., 2002). In general, the amount of chlorophyll in water samples can be used as a measure of the concentration of suspended phytoplankton. In addition to this it can be used as an indicator that a system is vulnerable to eutrophication (Ferreira et al., 2011). The highest chlorophyll a concentrations of up to 22.2µg/L on 14/6/13 were detected at the Iford Bridge site on the River Stour. In addition to this, the River Stour was generally higher in chlorophyll a than the River Avon. This is most likely due to the increased nitrate and phosphate concentrations in the River Stour compared to the River Avon (Schindler et al., 1971; Reimann et al., 1989; Statham et al., 2012) (Figures 2.6 and 2.8). Increased chlorophyll a concentrations at Iford Bridge compared to Throop suggest that nutrients released from Holdenhurst sewage treatment works were increasing phytoplankton growth. The chlorophyll a concentrations at the estuarine site at Mudeford Quay show similar spikes as in the rivers but also show consistently higher chlorophyll a concentrations over the summer months than either of the rivers. This indicates that the estuary has a distinct phytoplankton community compared to the rivers. This effect has been observed in other studies (Malone et al., 1988; Gypens et al., 2013).

2.4.2 Nitrate

Human activity has doubled the loading of biologically available nitrogen to the terrestrial biosphere compared to pre-industrial levels (Galloway et al., 2004; Schlesinger et al., 2006; Seitzinger et al., 2006). Increased riverine fluxes of nitrate can cause numerous problems, such as harmful algal blooms and as a contaminant in drinking water due to its harmful biological effects so understanding fluxes of nitrogen is vital for future macronutrient modelling. Mean annual fluxes of nitrate were similar at Knapp Mill and Throop, despite the higher flow rate at Knapp Mill. This was due to higher baseline concentrations of nitrates in the River Stour as opposed to the River Avon. Fluxes of nitrate were increased by increased river flow rate at both sites, and the maximum flux of nitrates was from December 2013 to March 2014, this is despite lower concentrations of nitrates in the river sites. This is due lo large volumes of water transferring nitrate downstream at high rates (Herman et al., 2008).

Howden and Burt (2009) found that there was a sustained linear increase in nitrate concentrations in two rivers in Dorset, UK between 1965 and 2007. In addition to this, chalk aquifers may mean that water with elevated nitrate concentrations takes many years to reach the surface. This may mean that any changes in farming practices to limit nitrate pollution will have a very long response time in Chalk catchments a and may not be measurable for many years. In this investigation, the river Avon is groundwater fed, with chalk aquifers, so any nitrate pollution could take many years to be removed once polluted (Neal et al., 2006).

Nitrate concentrations at the three riverine sites were highest in March 2014, after the period of high flow. This may be due to the application of fertiliser or slurry to fields in spring, which the UK government recommends in order to reduce loss by leaching (Nutrient management plan, 2009). In addition to this, the diluting effect of the high river flow was reduced as the river flow decreased, contributing to the elevated nitrate concentration. The River Avon had lower nitrate concentrations than the River Stour over the sampling period, as well as historically by comparing the data from the Environment Agency. The mean annual concentration of nitrate at Knapp Mill was 117µM lower than at Throop on the River Stour. One main reason for this may be the fact that the River Avon is groundwater fed from chalk aquifers, which can buffer nutrient inputs from land run off. However, Since the 1970s, nitrate (NO3) contamination of groundwater has become a significant environmental problem and groundwater based systems are vulnerable to significant nitrate enrichment due to human application of fertilisers, STW run off and septic tank effluent (Rivett et al., 2008). Long term monitoring of groundwater-fed rivers such as the River Avon is vital to further understanding of nitrate enrichment of chalk aquifers.

Nitrate concentrations appear to be affected by point source inputs into the River Stour between Throop and Iford from the outflows from Holdenhurst sewage treatment works. The River Avon at Knapp Mill showed more constant and lower nitrate concentrations over the sampling period than at Throop and Iford on the River Stour.

The nitrate concentration at Mudeford Quay was less than the sum of the nitrate concentrations at Iford and at Knapp Mill. This indicates that there is nitrogen removal within the estuary. This may be due to denitrification within the estuarine sediments by bacteria and other microorganisms (Texieira et al., 2010). In addition to this nitrification rates decrease with salinity (Giblin et al., 2010), so the saline nature of the estuary may be having an effect. Nitrate can also be reduced to ammonium by dissimilatory nitrate reduction (DNRA) by certain organisms, and has been shown to be significant in temperate estuaries (Dong et al., 2011).

2.4.3 Phosphate

The seasonal pattern in SRP concentration at the river sites appears to be principally driven by the hydrology with high SRP concentrations coinciding with low flow periods in summer and autumn, and low SRP concentrations during the high flow winter and spring periods, suggesting dilution of point sources, particularly on the Stour, and of diffuse run off from farmland. This is further evidenced by a reduction in SRP concentration on 30/10/13 at all sites, which coincide with a short period of raised river flow.

The EU Water Framework Directive (WFD: CEC, 2000) provides guidelines for the water quality of a river based on the concentrations of various constituents (See Introduction, section 1.1). The WFD guidelines for phosphorus take into account the alkalinity and elevation of the sample site of the river, with the guideline standards increasing with decreasing elevation and increasing alkalinity (table 2.4). All four sites in this investigation fall within the ‘high alkalinity’, ‘lowland’ category on this scale, as the typical alkalinities are higher than 50mg/L and all sites are below 50m (Whitehead et al., 2014). With regard to phosphorus, the River Stour at Throop and Iford is classified as poor to moderate for the majority of the year, excepting the high flow period in early 2014. The River Avon at Knapp Mill is classified as high to good with regard to SRP and Christchurch Harbour at Mudeford Quay is moderate (Table 2.4).

Table 2.4: Boundaries for water quality classifications as described by the EU Water Framework Directive (2000). The River Stour and Avon both fall into the 'Lowland', 'high alkalinity' category.

Type	Annual mean of reactive phosphorus (µM)			
	High	Good	Moderate	Poor
Lowland*, low alkalinity **	0.6 (0.4-0.8)	1.3 (0.9-1.7)	3.7 (2.8-4.5)	27.2 (24.3-29.6)
Upland*, low alkalinity **	0.4 (0.4-0.6)	0.9 (0.9-1.3)	2.8 (2.8-3.8)	24.3 (24.3-27.5)
Lowland*, high alkalinity**	1.2 (0.9-1.6)	2.2 (1.7-2.9)	5.6 (4.5-7.0)	32.3 (29.7-35.4)
Upland*, high alkalinity**	0.8 (0.6-1.2)	1.6 (0.9-2.3)	4.3 (3.5-5.7)	29.0 (26.7-32.6)

*"Lowland" means less than or equal to 80 metres above mean sea level and "Upland" means more than 80 metres above mean sea level.

** "Low alkalinity" with a concentration CaCO3 of less than 50 mg per litre and "High alkalinity" with a concentration CaCO3 of greater than or equal to 50 mg per litre

The phosphate concentrations in the River Stour and Avon over the sampling period show a clear relationship with river flow rate (Appendix 2). The highest SRP concentrations occurred during low flow conditions, with all concentrations over 10µM occurring at flows under 20m^3/s. These high SRP concentrations decreased with increasing flows with no points greater than 5µM when flows were over 20m^3/s at Throop and Iford. This suggests dilution of the riverine phosphorus by the large river flow originating from high rainfall (Arreghini et al., 2005). However, there were a few high SRP concentrations that coincided with high flow periods, including two data points on 15/01/14 and 23/01/14 at Knapp Mill. These two points are potentially associated with a hysteresis effect after the large storm event in early 2014, probably due to dry conditions prior to the storm allowing large quantities of phosphorus to accumulate within the floodplain (Bowes et al., 2015).

During low river flow periods, phosphate levels were highest, and more variable, which is indicative of point source inputs into the rivers (Jarvie et al., 2005). This variation was lower at Knapp Mill on the River Avon than at Throop and Iford on the River Stour, indicating that the River Stour is more affected by point source inputs than the River Avon. This is clearly the case at Iford Bridge as the difference between high and low flow conditions is quite distinct, and there are frequent increases in phosphate concentration seen at Iford but not detected at Throop, most likely due to the outflow from Holdenhurst STW just upstream from Iford.

A major factor affecting the flux of phosphate into coastal waters is resuspension of sediment, notably during high flow events. Corbett (2010) found that during resuspension events, fluxes of phosphorus could be increased up to 15%. Similar results were seen in this investigation, with fluxes of N, P and Si all elevated during high flow events, with a spike in concentration at the start. This is most likely due to a resuspension event, releasing benthic phosphorus, which had been stored during the preceding low flow periods.

2.4.4 Silica

The Silica concentrations at the sample sites in this investigation appear to be altered by hydrology, with a reduction in silica concentration during periods of high flow, likely due to dilution (Sigleo and Frick 2003). However, there may also be a biological factor, especially at Knapp Mill. The more dramatic reduction in silica during the high river flow period in early 2014 at Knapp Mill (Figure 2.9) in relation to concentration changes at Throop on the River Stour may be due to removal of silica up stream or by benthic diatom growth in shallow parts of the river, up stream of the sample sites (Figure 2.2). This increase in chlorophyll a concentration is not seen at Throop and may have led to silica removal from the water column via biological uptake (Conley and Malone, 1992). This indicates that there is likely to be removal processes within the river for silica such as removal by siliceous phytoplankton such as diatoms. A strong negative relationship between silica concentration and salinity was observed at Mudeford Quay (Appendix 2) with two distinct lines of scatter suggesting the two main river sources have different freshwater end member concentrations. Similar observations identified in previous studies such as Bell, (1994), indicated conservative mixing behaviour of silicate in estuarine systems.

2.4.5 Historic nutrient data

The long-term monthly sampling of the River Avon at Knapp Mill and the River Stour at Throop by the Environment Agency allows comparison of the inorganic nutrient concentrations obtained in this investigation with previously recorded inorganic nutrient concentrations.

There is a noticeable long-term trend of reduction in phosphate concentration in the River Avon at Knapp Mill since 2000. This may be due to a number of reasons, for example, phosphates have long been used in detergents as a water softener, which can enter rivers via drainage. In 2004 the European Union implemented regulation to require biodegradability in all detergents, and limited phosphates in detergents in 2013 with plans to further reduce phosphates in detergents in 2017 (Council of the European Union, 2012). This may have led to a reduction in the phosphate delivery to the rivers. In addition to this, the implementation of tertiary sewage treatment on the River Avon (DEFRA, 2002) may have reduced the phosphate load into the river.

The comparison between the monthly sampling of the sites by the Environment Agency and the weekly sampling undertaken during this investigation indicates that the weekly samples can identify nutrient dynamics which are not detectable from monthly resolution data. Recently, research has focused on higher frequency nutrient data from rivers, in order to study nutrient behaviour on a more frequent timescale (Bowes et al, 2015; Bieroza and Heathwaite, 2016; Lloyd et al., 2016). Automated analysers employing lab on chip technology can be used to analyse nutrients in-situ (Beaton et al., 2012). However, there are some issues with this type of automated sampling, which can cause problems in analysis. A major issue with automated samplers is difficulty with filtration, which restricts the fractions of phosphorus that can be analysed. In addition to this, Bieroza and Heathwaite, (2016) found that accurate detection of low P concentrations was challenging due to large errors associated with the in situ measurements. Automated samplers can also be used to collect and store samples remotely. The samples can be picked up in batches and returned for laboratory analysis. However, this method of sampling can mean that sampled water is left in the sample bottles for a week or more prior to collection, increasing the risk of changes due to biological activity or sample contamination. This means that extra steps such as adding a small volume of poison to the sample bottle prior to sampling need to be undertaken. Therefore, there is a balance when analysing nutrients from rivers between obtaining high frequency but low precision data or higher precision data but analysed at a lower frequency (Bieroza and Heathwaite, 2016).

2.4.6 Fluxes of nutrients

Fluxes of nitrate, silica, phosphate and nitrite at Throop and Knapp Mill were characterized by elevated fluxes during high periods of river flow followed by a return to the typical flux values during low base flow conditions. Over the period of 9/12/13 to 13/3/14 (9 weeks), 31.2% of the total SRP flux at Knapp Mill and 36.9% at Throop and 34.6% at Iford (58 weeks at Knapp Mill, 57 at Throop and 44 at Iford) (Figures 2.10 and 2.11) was transported through the sites. High fluxes of inorganic nutrients associated with high river flow have been observed in a number of different studies (Royer et al., 2006; Alexander et al., 2007; Holmes et al., 2012). This indicates that fluxes are highest during elevated winter flows but then decrease in spring and summer mostly due to the combined effects of reduced river flow rates plus dilution and biological uptake of nutrients (Holmes et al., 2012).

The flux of phosphate was more variable at Throop than at Knapp Mill, most likely due to the a clay-dominated catchment system of the River Stour as opposed to the groundwater fed, chalk based River Avon. In addition to this, a lower % of the flux of SRP occurred during the high flow event in early 2014 at Throop. Even so, the flux of phosphate at Iford was low over the summer and increased during high rainfall events. The total flux of nitrate and phosphate over the year is higher through the River Stour than the River Avon. This means that despite the lower flow rates in the River Stour than the River Avon, particularly during summer, the consistently high phosphate concentrations in the Stour contribute a higher flux of phosphate to the estuarine environment than the River Avon. This also reinforces the need for increased treatment of effluent from STW's if estuarine water quality targets are to be met (Jarvie et al., 2006).

Silica flux also increased in response to high river flow at both Knapp Mill and Throop. However, the silica flux returned to close to the summer levels at Knapp Mill much sooner than at Throop, where the silica flux remained elevated until April 2014. This may be due to a number of reasons including differences in catchment geology and reduced biological activity compared with the River Avon.

2.4.7 Conclusions

The dynamics of inorganic nutrient concentrations in rivers are complex and show variation on a daily basis as well as on a decadal time scale. The variation in both nutrient concentration and changes in river flow rates together influence the flux of nutrients from a river system into an estuary. Estimates of nutrient fluxes are generally based on water samples collected by the EA at weekly intervals at best where as river flow data is collected on daily or even hourly frequencies. Water samples collected on a daily basis should greatly improve the estimate of nutrient fluxes although this is only feasible by using an automated water sampler. Results from this investigation reinforce the need for high-resolution sampling in the future, at a minimum of weekly intervals. In-situ autoanalysis will be vitally important in analysing nutrient dynamics at a much higher resolution, as it is clear that nutrient concentrations in rivers are dynamic and variable on short time scales. This investigation highlights weekly changes in inorganic nutrient concentration, in particular the effects of high river flow on the fluxes of nutrients. Future legislation must focus on reducing nutrient delivery to rivers over winter and during high river flow, as this is when the majority of nutrient flux to coastal systems occurs. Point source inputs of inorganic nutrients are evident on the River Stour so legislation such as the reform of sewage discharges from septic tanks (DEFRA 2015) and the EU target to reduce phosphate concentration in lowland rivers are vital to the future health of aquatic ecosystems.

Chapter 3: Optimization of the determination of dissolved organic phosphorus in environmental water samples using a combination of ICP-MS and spectrophotometric colourimetric analysis.

3.1 Introduction

Most methods for analysis of inorganic phosphorus concentrations in water samples are based on the spectrophotometric detection of molybdenum blue (Monaghan and Ruttenberg, 1999; Jarvie et al., 2002b; Rinker and Powell, 2006; Cai and Guo, 2009; Yates and Johnes, 2013; Worsfold et al., 2016). This coloured complex is formed by reaction of phosphate with acidified molybdate, producing 12- molybdophosphosphoric acid, which is subsequently reduced to phosphomolybdenum blue (McKelvie et al., 1995), for example:

$$PO_4^{3-} + 12MoO_4^{2-} + 27H^+ \rightarrow H_3PO_4(MoO_3)_{12} + 12H_2O$$

$$H_3PMo(VI)_{12}O_{40} + Reductant \rightarrow [H_4PMo(VI)_8Mo(V)_4O_{40}]^{3-}$$

'Molybdate reactive' phosphorus in the dissolved fraction is commonly referred to as soluble reactive phosphorus (SRP) or filterable reactive phosphorus because this fraction may contain some acid labile molybdate-reactive organic phosphorus species (McKelvie et al., 1995; Baldwin, 2013). Despite this, SRP is still the most widely used surrogate of inorganic phosphorus because it is practically convenient to measure (Worsfold et al., 2016).

The most widely used method to analyse SRP is that of Murphy and Riley (1962). This involves the use of ascorbic acid in the presence of potassium antimonyl tartrate. The presence of potassium antimonyl tartrate enhances the rate of formation of molybdenum blue (although it does not act as a catalyst) and Sb is incorporated into the complex as [$PSb_2Mo_{12}O_{40}$] which has a $\boldsymbol{\lambda}_{\mathbf{max}}$ at 880 nm.

The chemical determination of TDP requires that a sample must first be digested to convert all organic and colloidal P forms to detectable inorganic phosphate before analysis using the previously described molybdenum blue assay. There are a number of techniques that can be used for the digestion of TDP to SRP. The most commonly used chemical technique is by the addition of an oxidising agent, such as potassium perchlorate, in an acidic or alkaline medium, at high temperature.

The alkaline/acid digestion procedures have been shown to deliver good total phosphorus recoveries (0-100%) from standardised reference materials up to 3.2µM P (Lambert and Maher, 1995), however, a dilution step was recommended for any samples with TP concentrations higher than this. Dayton et al., (2017) compared three persulphate digestion techniques on soil suspensions, (Acid Persulfate, Alkaline Persulfate and a technique used by the United States Geological survey (USGS)), in which an alkaline/acid persulfate digestion (starts out alkaline and ends at an acidic (<2.2) pH due to the thermal degradation of persulfate to sulphuric acid). The Acid Persulfate method was most effective, with an average total phosphorus recovery of 96.6%. The second most effective method was the USGS technique with an average total phosphorus recovery of 76.1%. However, the Alkaline Persulfate method was the least effective with an average 24.5% total phosphorus recovery.

Other oxidising techniques include using UV photo-oxidation to convert organic forms of P into SRP either alone (Peat et al., 1997) or with a thermal digestion (Perez-Ruiz et al., 2001). However, this technique can be ineffective at oxidising compounds such as ATP and Polyphosphates (Ormaza-Gonzalez and Statham, 1996).

Another method to analyse total phosphorus is using inductively coupled plasma mass spectrometry (Maher et al., 2001; Ivanov et al., 2010). However, ICP-MS as a technique has not been widely implemented for analysis of total phosphorus, mainly due to high concentrations of P being required in the sample (Lindeman et al., 2007), and due to interferences from $^{14}N^{16}O^{1}H^{+}$, $^{15}N^{15}N^{1}H^{+}$, $^{15}N^{16}O^{+}$, $^{14}N^{17}O^{+}$, $^{13}C^{18}O^{+}$ and $^{12}C^{18}O^{1}H^{+}$(Corey et al., 1986; Reed et al., 1994; Tan and Horlick, 1986). Due to the formation of these molecular ions inside the argon plasma, the detection of phosphorus and sulphur is significantly disturbed. Using an ICP-MS with a quadrupole mass analyser requires a collision cell in order to reduce the effect of these interferences. This technology enables the sensitive determination of elements normally affected by interferences such as phosphorus or sulphur, based on the chemical or physical resolution of the elements of interest from the interfering polyatomic ions generated in the argon plasma due to the application of reactive gases or kinetic energy discrimination (Pröfrock et al., 2004). Collision–reaction cell technology is currently the most accepted technique to effectively overcome spectral interferences in ICP-MS analysis. However, the use of high resolution ICP-MS (HR-ICP-MS) can also remove the effects of these interferences and resolve a single peak for ^{31}P (Regelink et al., 2013) without the need for a collision cell. For example, Ivanov et al., (2010) successfully quantified TP in digested biological plant tissue after a pre-digestion with hydrofluoric acid. HR-ICP-MS will quantify the total phosphorus concentration but will not give any additional information on the composition of the phosphorus compounds in the sample. Recent research using HPLC-ICP-MS has successfully separated and quantified a range of different phosphorus compounds (Heerboth, 2007; Atlas et al., 2015). However, this method is less sensitive when used in conjunction with a quadrupole mass analyser and collision cell (ThermoScientific, 2017). HR-ICP-MS is more suitable than a quadrupole-collision cell ICP-MS for ultralow detection of non-interfered elements and for elements that require low to medium resolution (e.g. a mass resolution of between 400 and 4000) (ThermoScientific, 2017)

The aim of this chapter is to develop and optimise a method to analyse total phosphorus in an environmental water sample using HR-ICP-MS and to combine this with a measurement of SRP to calculate by difference the dissolved organic phosphorus concentration. This chapter outlines the methodology and the troubleshooting steps that were taken while developing this method.

3.2 Methods

3.2.1 Sample collection

Water samples were collected from four sites, at Throop and Iford on the River Stour, at Knapp Mill on the River Avon and at the mouth of Christchurch Harbour at Mudeford Quay (See chapter 1). At each site, a water sample of about 1 litre was collected from the surface using a pre-rinsed bucket and then decanted into pre-rinsed, 1L wide-mouth, polyphenylene ether (PPE) bottles. These were then transferred in a cool box back to the laboratory. Samples were collected on a weekly basis from 25/4/13 until 7/5/14, and then on a fortnightly basis from 7/5/14 to 13/8/14 at Knapp mill, Throop and Mudeford Quay. Samples from an additional site at Iford on the River Stour 3.6km downstream from Throop were collected on a weekly basis from 6/6/13 to 8/4/14. At each site, a YSI EXO2 Multiparameter Sonde (Xylem analytics) was deployed, and measurements of the temperature (°C), conductivity (mS) or salinity (PSU), chlorophyll a (μg/L), dissolved oxygen (mg/L), oxygen saturation (%), pH and total depth (m) were recorded.

3.2.2 Filtration and storage

On return to the laboratory water samples were filtered on the same day as collected to minimise any chemical or biological changes to the sample. A sub sample of 65ml was immediately passed through a pre-combusted Whatman GF/F glass microfiber filter (retains particles down 0.7µm) and 20 ml added to a clean plastic vial to which 5μl of a 4g L^{-1} solution of Mercuric Chloride was added. These samples were later used for the analysis of inorganic nutrients on a multi channel continuous flow autoanalyser at the University of Portsmouth.

A separate sample was also prepared for analysis of TDP using ICP-MS, and SRP using spectrophotometric colourimetry. Two 60ml samples were passed through a pre-combusted Whatman GF/F filter and transferred into two separate 75ml HDPE bottles. These samples were labelled and then kept frozen at -20 °C prior to analysis.

3.3 ICP-MS Methods and method development

3.3.1 Instrumental

ICP-MS can been used to analyse the TDP concentration in an aqueous sample (Cantarero et al., 2002; Ivanov et al., 2010). To prepare a sample for ICP-MS analysis, the sample had to be diluted at least 20x with 3% HNO_3 due to the solubility of the nitrate present in the samples as well as its oxidising ability and the relative freedom from chemical and spectral interferences as compared to acids containing Cl, S or F. 1ml of sample was weighed into an acid washed (10% HNO_3 for 24 hrs) 5ml HDPE vial. An internal standard was then added and the solution was made up to 5ml with 3%HNO_3.

A Thermo Element XR HR-ICP-MS (University of Southampton, Earth and Ocean Science) was used which incorporates an electromagnet and an electrostatic analyser (ESA) with a reverse double-focussing mass spectrometer with a reverse Nier-Johnson geometry (the ESA is located behind the magnet).

The steps in setting up the HR-ICP-MS for analysis are as follows: The instrument is switched on and allowed to settle for at least 30 minutes. A 1ppb tuning solution of 1ppb indium and 1ppb uranium is added, and the sensitivity of the instrument is checked, (U should be around 200,000-250,000 counts). The uranium:oxide ratio is then adjusted to <5%, and the stability is checked on a rate meter. This is done by moving the torch further from the cone or reducing the input gas volume. An analytical sample sequence was then written and programmed into the ICP-MS, a typical sequence order is shown in (table 3.1).

The resolution of HR-ICP-MS can be changed by adjusting the width of the entrance and exit slits into the spectrometer. In this investigation, phosphorus was measured at medium resolution because the ^{31}P peaks can be resolved from the aforementioned interferences ($^{14}N^{16}O^{1}H^{+}$, $^{15}N^{15}N^{1}H^{+}$, $^{15}N^{16}O^{+}$, $^{14}N^{17}O^{+}$, $^{13}C^{18}O^{+}$ and $^{12}C^{18}O^{1}H^{+}$) at this resolution. Using high resolution mode would have been unnecessary as with an increase in resolving power, there is a subsequent decrease in signal intensity, resulting in a reduction in accuracy of measurements (Ekman, 2009). At low resolution ^{31}P cannot be resolved and at high resolution the sensitivity of the ICP-MS instrument is reduced.

Table 3.1: Typical Order of HR-ICP-MS Analysis in a measurement period

Analysis Order	Sample
1	Unspiked 3% HNO_3 blank x 3
2	HNO_3 blank spiked with 1ppb Sc and In and 5ppb Be
3	Phosphorus standards
4	Sample run (3 repeats)
5	Phosphorus Standards

At this point the mass offsets (required for MR and HR measurements) were checked and adjusted if necessary.

A simple sample introduction configuration was used for all P analyses, namely a PFA Peltier-cooled cyclonic spray chamber (ESI) with a PFA nebuliser being pumped (at about 100ul/min) via peristaltic pump. The system used a Ni H-skimmer cone and Ni standard sample cone. Samples were introduced via an autosampler with a HEPA filtered enclosure (ESI SC2). The ELEMENT method used the acquisition parameters shown in table 3.2:

Table 3.2: Acquisition parameters for the HR-ICP-MS method used in this investigation

Analysis Order	Sample
Mass window	125
Sample time	0.02s/sample
Samples per peak	40

Using the ELEMENT operating software, an analytical sequence was constructed containing blanks, calibration standards (derived from synthetic standards, (table 3.3) reference materials (Section 3.3.4) and the unknown samples). All solutions were spiked with an internal standard (Section 3.3.2). Data were acquired for the isotopes of interest in peak-jumping mode (4 x 40 second repeats per sample). After each sample analysis, a wash solution containing 3% HNO3 was run until background levels of all elements were achieved (typically <3 minutes). The data quality was monitored throughout the run by examination of the statistics produced after each analysis. Within run reproducibility was typically better than 2% RSD for the 4 repeats. Any anomalous results were noted and re-run at the end of the procedure. The data handling procedure included the following steps: Internal correction, blank correction, calibration against 5 standards then dilution correction.

In addition to dilution, an internal standard was added to all samples to account for instrument drift. There are several guidelines to be followed when selecting an internal standard, including, avoiding isobaric interferences and molecular oxides and other molecular interferences. The naturally occurring concentration of the element used as an internal standard must be insignificant in comparison to the amount added, and the atomic mass of the internal standard elements should be as close as possible to that of the mass of the target element (Mantaser, 1998).

Because the objective of this investigation was to determine the concentration of phosphorus, the internal standards used were Beryllium, Scandium and Indium. Beryllium, and Indium are used because they are relatively low and high mass elements, respectively, and Scandium is used because it is close in mass to the target element of Phosphorus. All three of the internal standards were expected to be present in trace amounts in the natural water samples. This was confirmed by analysis of unspiked samples and standard addition experiments.

3.3.2 Standard addition

The main sources of errors that can diminish accuracy in ICP-MS analysis are interferences, from either spectral or matrix effects (Rodushkin et al., 1998; Abbayad et al., 2001). A matrix element is a high concentration species in the sample that affects the signals of other analytes. The method of standard additions is a valuable approach that can be used when the sample matrix cannot be readily reproduced. For complex matrix solutions, the methodology offers better accuracy than external or internal standards and is generally less expensive than isotope dilution. In most cases, a precision lower than 2% can be achieved (Cheatham et al., 1993). A known amount of analyte is added in a small 'spiked' volume directly to the unknown solution. The increase in signal per unit of added analyte is the slope of the calibration curve in the presence of the matrix. The initial concentration of analyte in the solution is determined by extrapolating the calibration curve to the blank signal, and noting the negative value on the concentration axis (Figure 3.6). The technique can be performed by either adding a single spike or multiple spikes to the sample solution (Abbayad et al., 2001).

A number of standard addition experiments were conducted on river water samples collected from Throop and Knapp Mill. An inorganic standard curve was created using the same phosphoric acid standard that was used in the analysis of SRP concentration (Table 3.3).

Table 3.3: Dilutions required to create the inorganic standard curve for ICP-MS analysis.

	Working standard 1	Working standard 2	Working standard 3	10ppb standard	5ppb standard	3ppb standard	1ppb standard	0.5ppb standard	0.1ppb standard
Concentration of inorganic P standard (ppb)	1001000	20000	1000	100	100	100	100	100	100
Target volume (ml)	20	20	20	20	20	20	20	20	20
Target concentration (ppb)	20000	1000	100	10	5	3	1	0.5	0.1
Dilution required	50.05	20.00	10.00	10.00	20.00	33.33	100.00	200.00	1000.00
Standard required (ml)	0.40	1.00	2.00	2.00	1.00	0.60	0.20	0.10	0.02
Dilutant required (ml	19.60	19.00	18.00	18.00	19.00	19.40	19.80	19.90	19.98

In addition to the inorganic phosphate standard curves, standard a sample from Knapp Mill was spiked with 1,2,3 and 4 ppb of inorganic P standard and a sample from Throop was spiked with 2,4,6,and 8 ppb of inorganic P standard (Figure 3.1). The added amounts were different because the River Stour at Throop typically has higher P concentration than the River Avon at Knapp Mill and it is preferable to at least double the P concentration after a number of additions. All samples were also spiked with In and Be as internal standards.

The two standard curves produced in this investigation show highly linear standard additions (Figure 3.1). The P concentration determined from the inorganic standard curve (Figure 3.1A) was 1.1ppb for the Knapp Mill sample and 6.4ppb for the sample from Throop. The Standard addition returned values of P of 1.14ppb and 6.04ppb respectively. This is a 5.1% difference at Knapp Mill and a 5.7% difference at Throop (Table 3.4).

Table 3.4: Concentration of a water sample from the River Avon and the River Stour derived from a standard addition and an external, inorganic standard curve.

Site	Concentration derived from inorganic standard curve	Concentration derived from standard addition	% difference
Knapp Mill	1.14	1.08	5.09
Throop	6.38	6.04	5.65

The difference between the phosphate concentrations determined from the standard curve and the standard addition at both sites was approximately 5% indicating that there may be a signal suppression effect of around 5% in the matrix of the environmental samples.

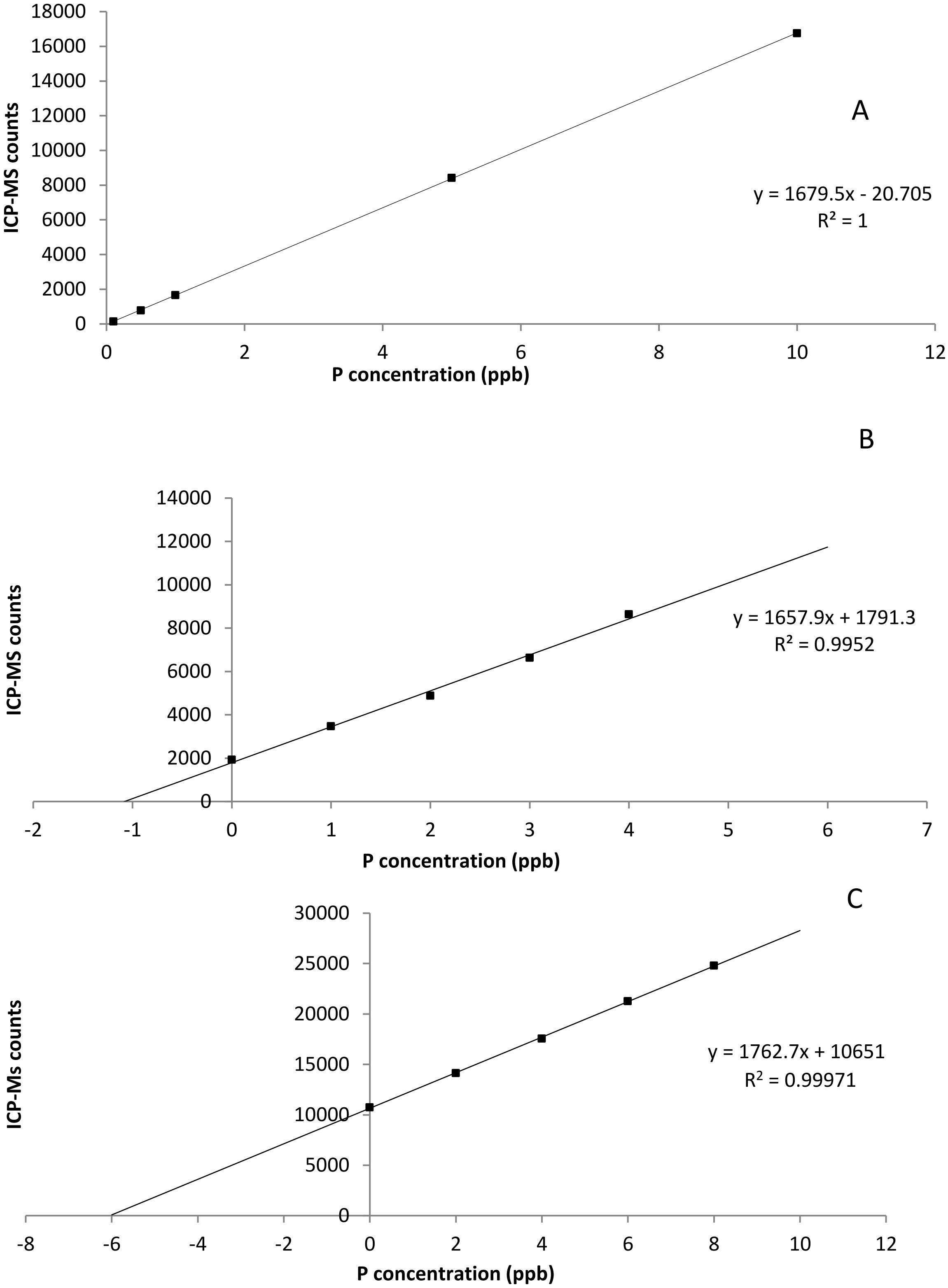


Figure 3.1: Inorganic standard curve (A), Standard addition of 1ppb of Avon river water (B) and standard addition of 2ppb for Stour river water (C).

3.3.3 Analysis of organic phosphorus standards using ICP-MS

It was important to measure known organic standards in order to assess the efficiency of the determination of TDP by ICP-MS. The organic phosphorus standard compounds selected were phytic acid ($C_6H_{18}O_{24}P_6$), a stable chemical used for the storage of phosphorus in plant tissues and glycerophosphate ($C_3H_7Na_2O_6P$), a labile compound found as a component of cell membranes. The standards were provided as solid powders which were diluted in 3% HNO_3 to create standard solutions of 10, 5, 1, 0.5 and 0.1ppb of phosphorus. These standards were spiked with the internal standard elements (Be, Sc and In) and analysed alongside the inorganic phosphoric acid standard.

The standard curve for the organic compounds were directly comparable with the inorganic standard curve (Figure 3.2) indicating that the plasma ionizes the organic compounds and is a viable alternative oxidation technique to the chemical methods. The relationship between the gravimetrically predicted TDP concentrations and analytically measured TDP of the two organic standards was linear and followed a 1:1 line (Figure 3.3), thus indicating that the ICP-MS analysis was efficiently analysing the TDP present in the sample.

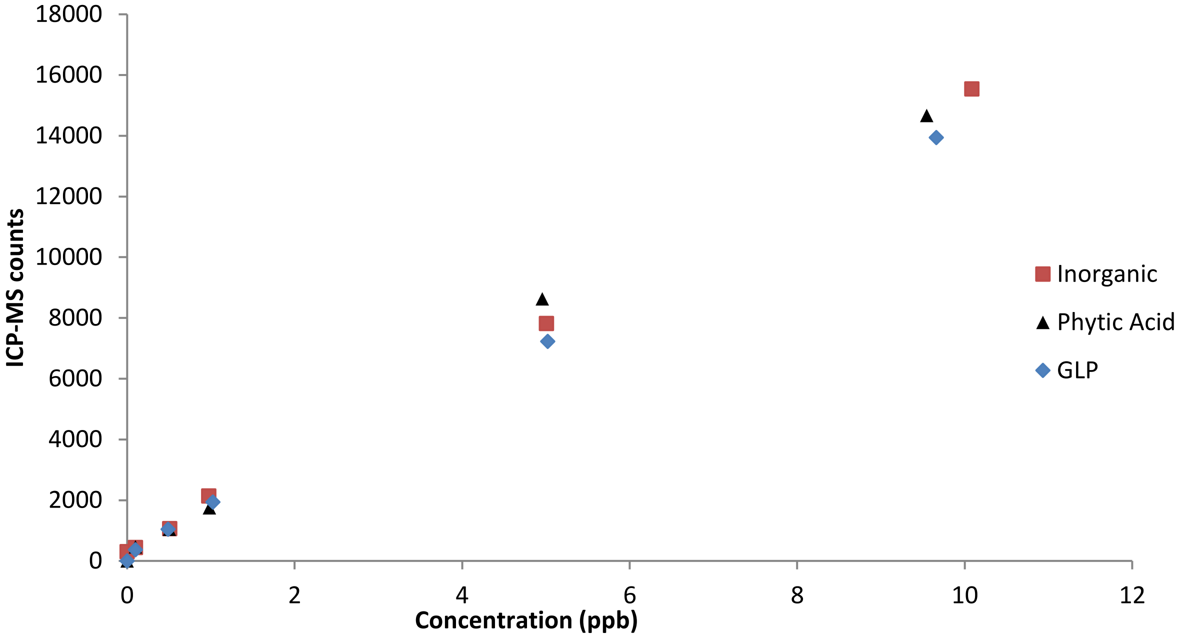


Figure 3.2: Standard curve comparison between phosphoric acid, phytic acid and glycerophosphate (GLP).

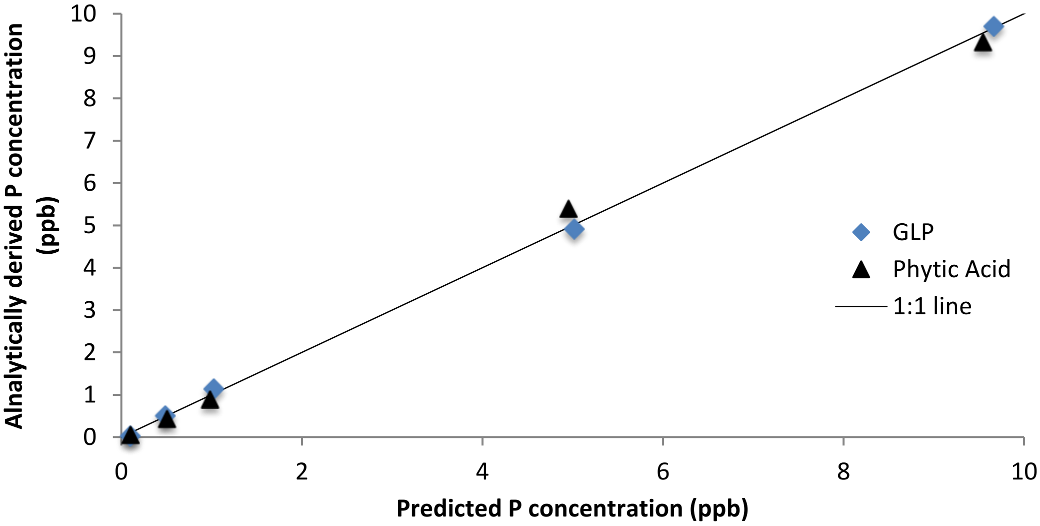


Figure 3.3: Standard curves of glycerophosphate (GLP) and phytic acid compared to a 1:1 line

3.3.4 Certified reference material for ICP-MS analysis

A certified reference material (CRM) (Fluka Analytical CRM QC1001) was obtained to assess whether the methods used were determining the correct concentration of total phosphorus. The CRM contains 7.52g/L total nitrogen, and 2.09g/L (2090 ppm) total phosphorus. The CRM was prepared for analysis by dilution by weight to a 15 ppm solution with Milli-Q , This was then diluted to a 100ppb working standard in 3% HNO_3 before dilution to 5ppb in 3% HNO_3 for analysis using ICP-MS (Table 3.5).

Table 3.5: Preparation of a CRM standard by dilutions from 2090 ppm to 100ppb.

	Working standard 1	Working standard 2	Analytical standard
Concentration of standard (ppb)	2090000	15000	100
Target volume (ml)	20	20	20
Target concentration (ppb)	15000	100	5
Dilution required	139.33	150.00	20.00
Standard required (ml)	0.14	0.13	1.00
Dilutant required (ml	19.86	19.87	19.00

Figure 3.4 shows the CRM with the gravimetrically derived expected P concentration and the analytically derived P concentration with 5 repeats. The expected P concentration is the concentration of the CRM, calculated in table 3.5 using gravimetric dilutions. The obtained P concentration is the concentration derived from ICP-MS analysis. As the CRM used in this investigation was an environmental sample, it was shown to be a mixture of inorganic and organic phosphorus, and therefore a difference between the TDP and SRP concentration was detected. However, no difference was detected between the expected concentration and the analysed TDP. The SRP concentration of the CRM was 2.4µM, indicating that 50.3% of the CRM is organic phosphorus. This analysis confirms that ICP-MS analysis is capable of determining

total phosphorus concentration in natural water samples to a high degree of accuracy.

Concentrations of the 5 repeated samples are shown in table 3.6.

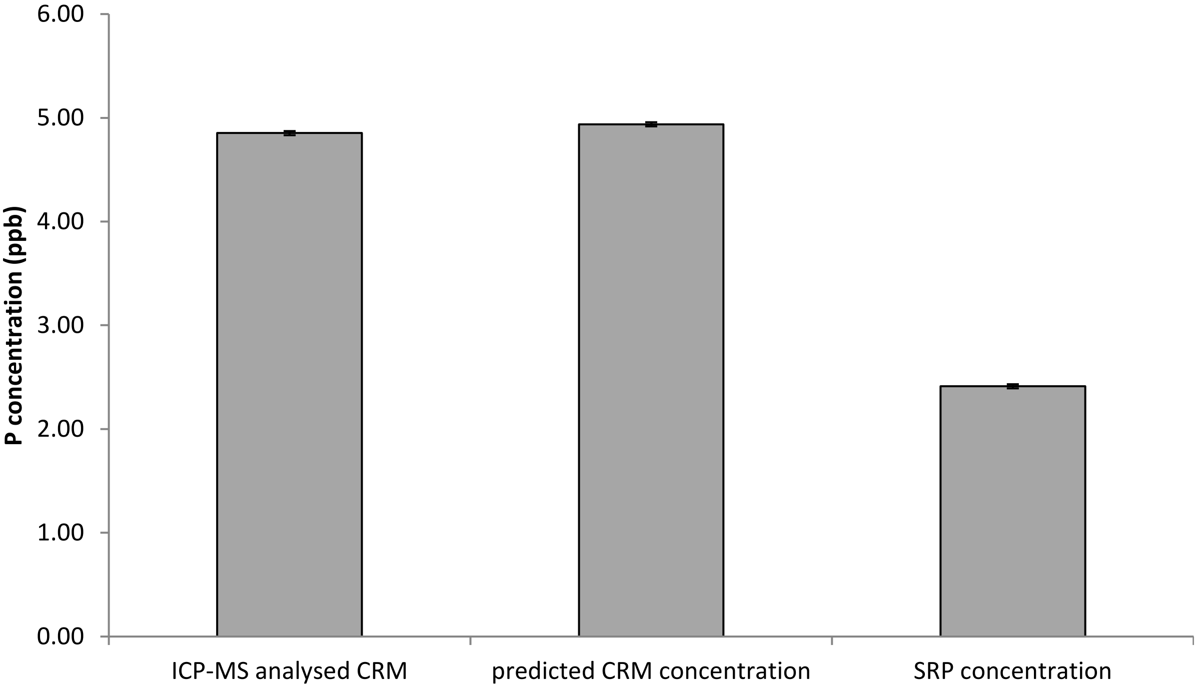


Figure 3.4: Analysed CRM concentration compared to the gravimetrically predicted CRM concentration and the SRP concentration of the CRM analysed using inorganic spectrophotometry. Error bars show 1 sd.

Table 3.6: Gravimetrically derived P and ICP-MS analysed P in a CRM in 5 repeated samples.

	P concentration ICP-MS (ppb)	Gravimetrically predicted P concentration (ppb)
CRM1	4.94	4.84
CRM2	4.84	4.99
CRM3	4.78	4.98
CRM 4	4.89	4.90
CRM 5	4.81	4.98
Average	4.85	4.94
Standard deviation	0.07	0.07

3.3.5 Effects of freezing the sample

Some form of rapid preservation is essential if large absolute and proportional changes in nutrient concentrations in collected natural water samples are to be prevented (Kotlash and Chessman, 1998). The samples analysed in this investigation were passed through a GF/F filter (0.7µm pore size) before being frozen to minimise biological activity (Gilmartin, 1967)

Initial analysis of SRP and TDP in defrosted riverine samples indicated that there may be interference from particles in the sample when analysed using ICP-MS. Because the analysed samples had been filtered prior to freezing any variation in the analysed concentration could be due to the freezing.

The effects of freezing and filtering samples were investigated using water from the River Avon, collected on the 19/12/13. Four different treatments were used on the water.

1. Unfiltered and unfrozen - sample was not altered in any way.
2. Unfiltered and frozen -sample was not filtered but was frozen.
3. Filtered and unfrozen - sample was filtered through a GF/F filter but was not frozen.

4. Filtered and frozen - sample was filtered through a GF/F filter before being frozen. Treatment number 4 is the most similar to the collected water samples from the 4 sites.

All samples were diluted 20x with 3%HNO_3 and were spiked with 5ppb Be and 1ppb In and Sc. They were then analysed using ICP-MS in conjunction with a standard curve and CRM to assess the total P concentration. Four replicates of each sample were analysed. Blanks were also frozen and filtered to assess what impact the filtration/freezing process has on the addition of phosphorus to the analysis. The blanks of Milli-Q water were exposed to either freezing or filtration in conjunction with the samples. 1ml of this blank was then diluted to 20ml in 3%HNO_3. All glassware was cleaned beforehand with 10% HCl.

Results from ICP-MS analysis show no difference in total phosphorus (TP) concentration between the unfiltered river water samples either frozen (4.05µM) or not frozen (4.02µM) (Figure 3.5). The TP concentration was, however, significantly greater in the unfiltered samples than in the filtered samples indicating that the ICP-MS method was detecting phosphorus associated with particles in the river water. There was also a larger standard deviation of the mean phosphorus concentrations detected in the unfiltered samples compared to the filtered samples indicating that the presence of particles in the sample may affect the precision of the ICP-MS analysis.

The filtered samples did show a difference in P concentration, with the unfrozen sample having a slightly higher P concentration than the frozen sample. This may indicate biological activity or release of P from particles during the period of time that the sample was left prior to analysis.

However, there was a much larger reduction in P concentration in samples which had been filtered, indicating that the main factor affecting the TP concentration in these samples was filtration, with freezing of the samples having a lesser effect. Blanks were also filtered, frozen and analysed using ICP-MS. Blanks had a slightly higher TP concentrations after filtration but by less that 0.1ppb, i.e. not enough to make a difference to the overall phosphorus concentration in the sample. The Milli-Q blanks were also very low in concentration (< 0.01µM TDP).

The results from this experiment indicate that, as expected, filtering a sample reduces the P concentration most likely due to the presence of particle-associated P (Paytan and McLaughlin, 2007). However, in both the unfiltered and filtered samples, freezing did not have a noticeable effect on the average phosphorus concentration in the samples. This indicates that the freezing regime employed in this investigation does not significantly alter the TDP concentration from the initial, unfiltered, sample.

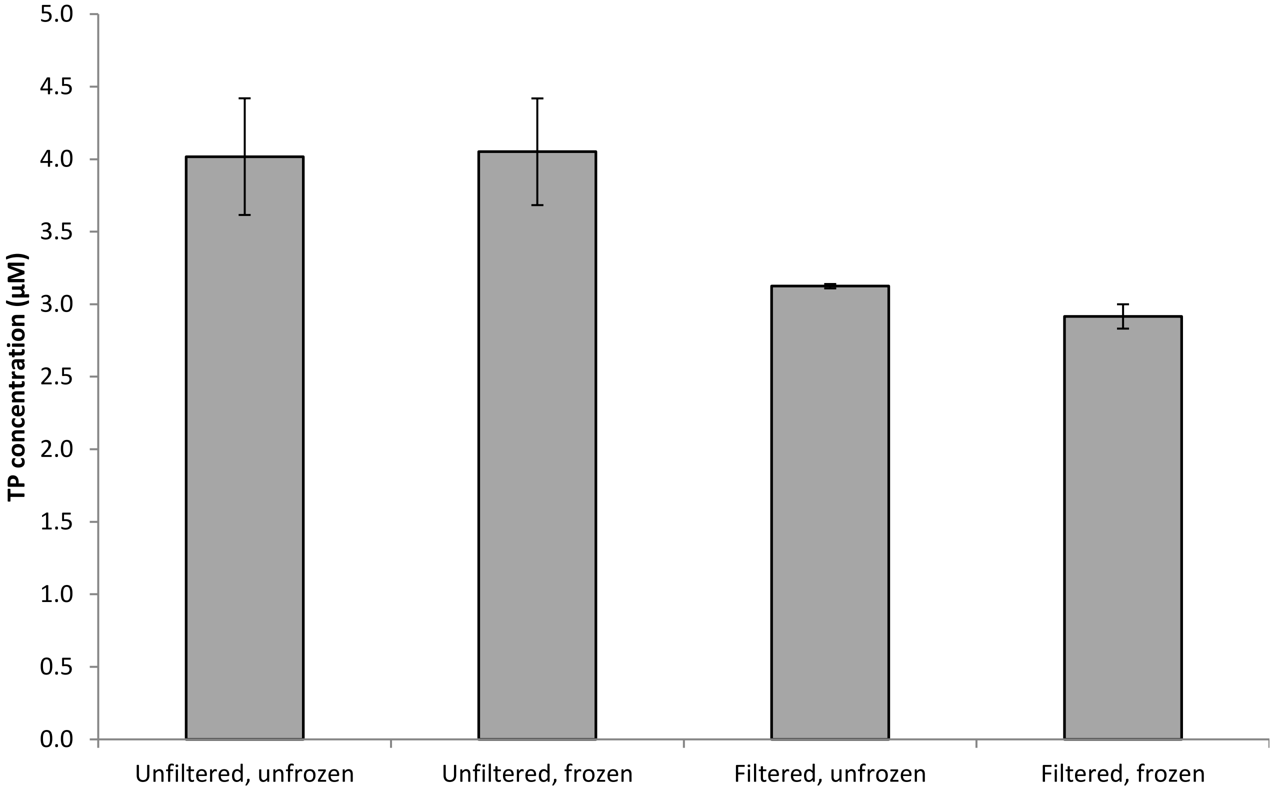


Figure 3.5: Total phosphorus concentration of samples following freezing and filtration. Error bars show 1sd. Each sample and blank was replicated 5 times.

3.3.6 Effects of filtration on sample

As the stored samples used in this study were filtered before analysis, a review of how filtration affects the sample was undertaken. Water was collected from the river Avon on 19/12/14, passed through a pre-combusted, 47mm GF/F filter (0.7µM pore size) and frozen. Samples were then defrosted and prepared for analysis. Once defrosted, samples were re-filtered through a pre-combusted 47mm GF/F filter (0.7µM pore size) before analysis using the spectrophotometric and ICP-MS methods explained above. In addition to the filtered samples, control samples where no filtration was used were also analysed. Each sample was replicated 4 times.

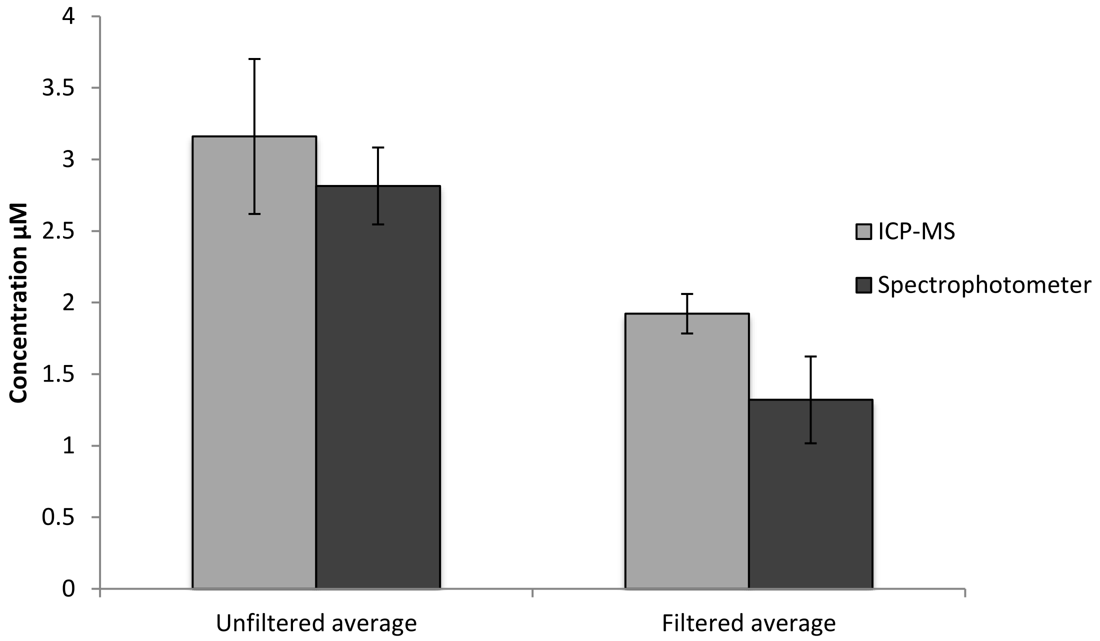


Figure 3.6: TDP and SRP of a filtered and unfiltered sample. Each sample was replicated 4 times. Error bars show ± 1 sd.

Figure 3.6 shows the phosphorus concentrations in filtered and unfiltered samples analysed using ICP-MS and colourimetrically. Results show, as expected, that the unfiltered samples contained more P than the filtered samples due to the association of P with particles. In addition to this, the error between samples was shown to decrease, again due to the reduction in particles. The unfiltered samples were higher in P, but no statistically significant difference could be found between the ICP-MS analysed concentration and the spectrophotometric analysed concentrations. The filtered samples were lower in P, but a significant difference between ICP-MS and spectrophotometrically analysed P can be detected.

3.3.7 Effects of chemical oxidation on water sample prior to ICP-MS analysis

The presence of particles in the water samples was hypothesised to potentially affect the TP signal using ICP-MS. Although the samples were filtered prior to freezing, coagulation of particles post filtration can occur during the freezing/thawing process (Nakamura and Okada, 1975) and these have the potential to interfere with the signal from the ICP-MS and spectrophotometric techniques.

Pre-digestion of the sample using potassium persulfate was used to investigate whether there was any difference in analytical efficiency when compared to a sample analysed using only ICP-MS. Water was collected from the river Avon on 19/12/14, passed through a GF/F filter and frozen. Samples were then defrosted and prepared for analysis.

There were four experimental conditions used for this experiment:

1. ICP-MS undigested
2. ICP-MS digested
3. Colourimetric analysis undigested
4. Colourimetric analysis digested

The addition of persulfate to the sample made no significant difference to the ICP-MS derived total phosphorus concentration (Figure 3.7). However, the standard deviation of the samples increased when persulfate was added. This indicated that the ICP-MS analysis of TDP did not benefit from a pre-digestion with potassium persulphate. The effect of persulfate on a sample analysed for SRP on the spectrophotometer was to oxidise a portion of the organic phosphorus into SRP, as expected. An organic phosphorus signal was evident but the high standard deviation of the two analyses reduced the precision with which this DOP signal can be quantified.

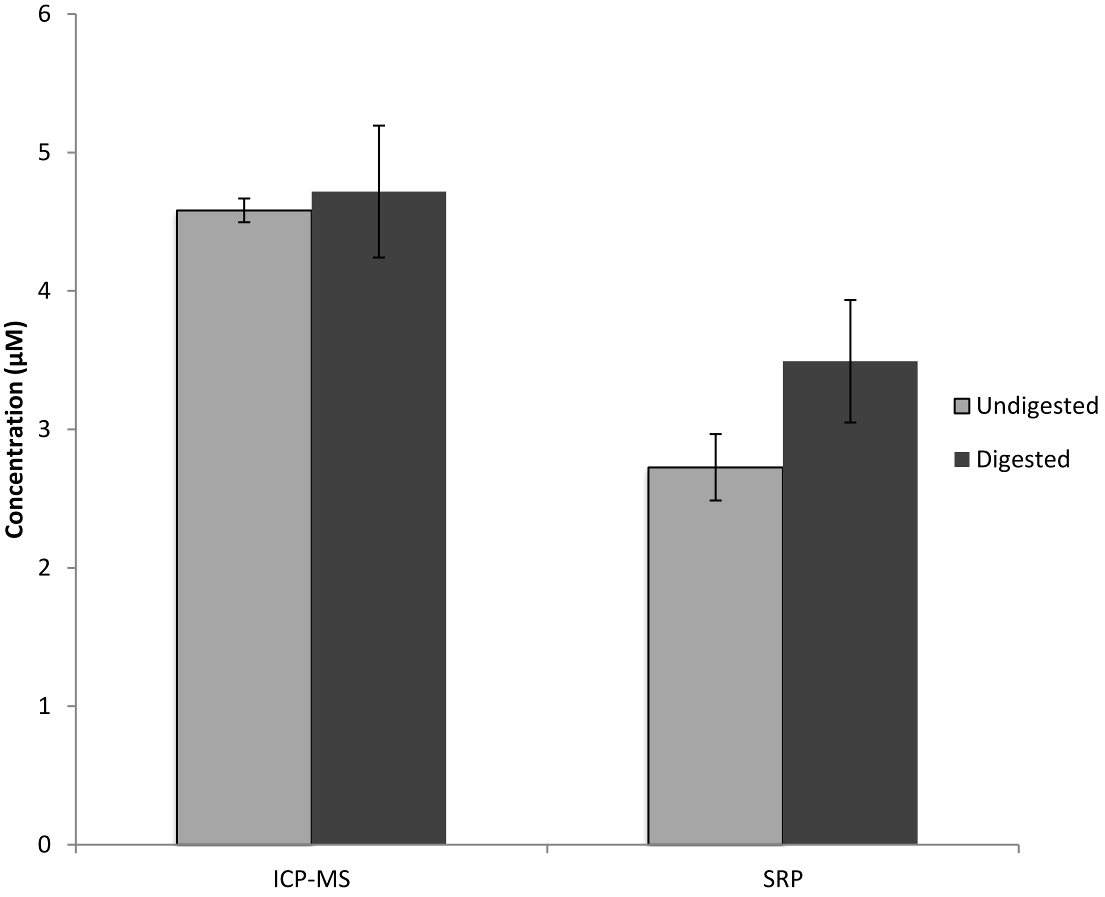


Figure 3.7: Phosphorus concentrations of digested and undigested filtered water samples analysed using ICP-MS and the SRP by spectrophotometer. Error bars show ±1sd.

3.4 SRP Methods

SRP is considered a fraction of the total dissolved phosphorus pool (Jarvie et al., 2002a) and here is assumed to be equivalent to the dissolved inorganic fraction of total phosphorus.

A 60ml pre-filtered sample was defrosted in a refrigerator overnight. A manual SRP analysis was then performed on the same day as the sample was defrosted to minimise change in the SRP concentration.

Analysis of SRP was performed using a variation of the molybdenum blue method (Murphy and Riley, 1962). A mixed reagent was made by mixing 25ml of 0.025M ammonium molybdate solution with 62.5ml of 12.5% sulphuric acid, 25ml of 0.3M ascorbic acid and 12.5ml of potassium antimonyl tartrate solution (0.34g of potassium antimonyl tartrate in 250mls Milli-Q). The above reagents were prepared every two months from solid chemicals and Milli-Q water (dilute sulphuric acid was made by adding 140ml of concentrated sulphuric acid to 900ml of Milli-Q water). Milli-Q water refers to 18.2 MΩ ultrapure water produced from a Milli-Q system through a filter size of 0.22 µm (Millipore, Merck, Germany). The mixed reagent was prepared fresh on the day of SRP analysis. 10 ml of the defrosted water sample was pipetted into a clean 20ml HDPE scintillation vial and 1ml of the mixed reagent added. HDPE vials were soaked in a bath of 10% HNO_3 for at least 24 hours before rinsing with Milli-Q, drying and storing ready for analysis. The sample was then left for one hour to allow for colour development. The sample was decanted into a 4cm path length, reduced volume glass cuvette and absorption measured at 885nm using a UNICAM 8625 spectrophotometer against a water blank. An external set of standard phosphate concentrations was prepared for every batch of samples analysed. A standard phosphoric acid solution was diluted to produce the series of standard solutions for both spectrophotometric SRP and ICP-MS analysis. This standard was diluted to produce a 20ppm working stock standard, which was made up monthly and was stable for 4 weeks when refrigerated. The working standard was then used to produce phosphorus standard curves by a series of dilutions. Table 3.7 shows an example of the dilutions required for standard solution preparation for colourimetric SRP analysis.

Table 3.7: Dilutions required to create the inorganic standard curve for colourimetric SRP analysis.

	Working Standard 1	20µM Standard	10µM Standard	5µM Standard	2.5µM Standard	1µM Standard
Concentration of inorganic P standard (ppb)	1001000	20000	20000	20000	20000	20000
Target volume (ml)	20	10	10	10	10	10
Target concentration (ppb)	20000.00	619.47	309.74	154.87	77.43	30.97
Dilution required	50.05	32.29	64.57	129.14	258.28	645.71
Standard required (ml)	0.40	0.31	0.15	0.08	0.04	0.02
Dilutant required (ml)	19.60	19.69	19.85	19.92	19.96	19.98

3.4.1 Automated Inorganic phosphate analysis

Water samples preserved with mercuric chloride (see section 3.3.2) were analysed for inorganic nutrients at the University of Portsmouth using a Seal Analytical AA3 segmented flow *autoanalyser (Seal Analytical*, Fareham, UK). Channels were set up for total oxidisable N (nitrate-N plus nitrite-N), nitrite-N, silicate and Soluble Reactive Phosphorus based on the colourimetric techniques of Henriksen and Selmer-Olsen (1970), Armstrong et al., (1967) and Murphy and Riley (1962) respectively. Total oxidisable N analyses were based on the hydrazine–copper reduction method which produces a reddish purple azo dye measured colourimetrically at 540 nm. SRP was determined colourimetrically following reaction with heptamolybdate and potassium antimony (III) oxide tartrate in acidic solution followed by reduction with ascorbic acid, producing a blue colour, measured colourimetrically at 880 nm. Silicate was analysed by adding an acidic solution of ammonium molybdate to a sample to produce silicomolybdic acid, which is then reduced to silicomolybdous acid (a blue compound) following the addition of stannous chloride. Tartaric acid is added to impede PO_4 colour interference.

3.4.2 Repeatability of the SRP analysis

The repeatability of the colourimetric analysis of SRP was investigated prior to the routine analysis of the stored water samples. Both unfiltered and filtered river water samples were analysed with 10 repeats using the colourimetric method. The samples were collected from the site at Knapp Mill on the River Avon on 19/12/14 and stored in a dark cool room. Milli-Q blanks were also analysed with 10 replicates. No standard curve was produced, as this test was just to assess the repeatability of the SRP assay.

The average absorption value of the filtered sample was less than the unfiltered sample (0.43 compared to 0.51) ($p = 0.000$, 9df) and the filtered blank was slightly less than the unfiltered blank (0.15 compared to 0.17) ($p=0.000$, 9df (Figure 3.8). However, the standard deviation of the filtered sample absorption as percentage of the mean was 3.2% compared to 4.1% for the unfiltered sample. The coefficient of variation of the filtered blank was 9.3% and for the unfiltered blank was 1.9%. These results indicate that the absorption standard deviation of a sample was lower after it had been filtered. However, the standard deviation of the blank was higher when filtered which may have been due to contamination during filtration. The low standard deviation of the mean absorption of 10 replicate water samples indicated that the method is sufficiently robust to be used for analysis of a large set of natural water samples.

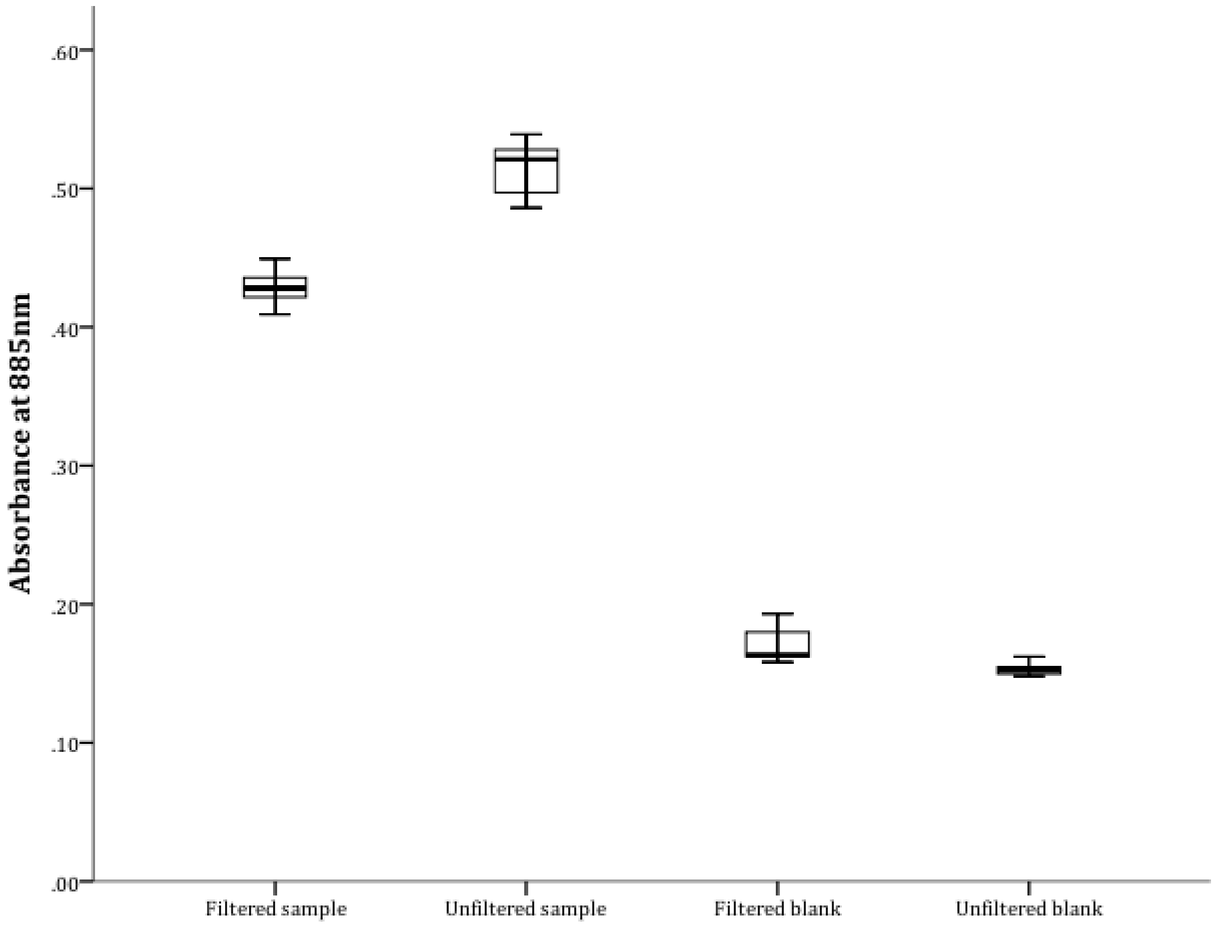


Figure 3.8: Absorbance at 885nm of filtered and unfiltered water samples and Milli-Q blanks. Each sample and blank was replicated 10 times with error bars representing +/- 1 standard deviation.

Standard curves were produced for every batch of water samples analysed for SRP. These were reproducible as indicated in Figure 3.9 showing the first 5 standard curves created for analysis of SRP. Additional standard curves were produced but not included in Figure 3.9 in order to make the figure clear.

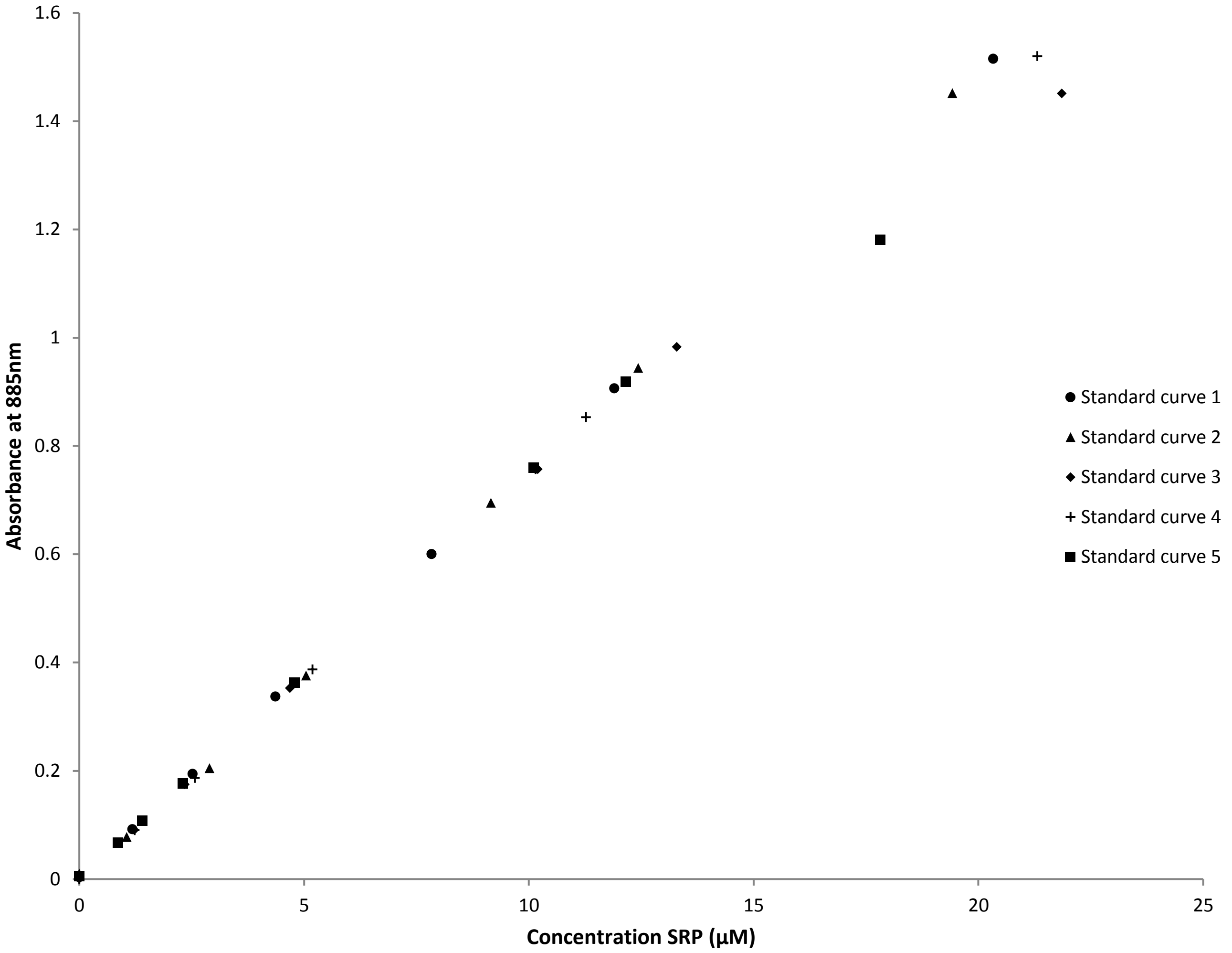


Figure 3.9: Standard curves from 5 sets of analysis of SRP with absorption measured at 885nm on a spectrophotometer. Each standard curve was created using similar standard dilutions by weight – see example in table 3.3.

3.4.3 Nonlinearity of SRP standard curve at high concentrations

The relationship between SRP concentration and absorbance at 885 nm becomes nonlinear above 20µM (Figure 3.10). According to the Beer- Lambert law, the relationship between absorption of light at a particular wavelength to the concentration of the substance that is absorbing the light is dependent on the path length of the cuvette used. In order to check if the reduction in absorbance at 885nm at higher concentrations of SRP was due to the cuvette path length, two cuvette sizes were compared; a 4cm path length cuvette and a 1cm path length cuvette. A standard curve was plotted for each cuvette and the same non-linear pattern was observed at SRP concentrations above 20 µM (Figure 3.10). This means that a simple linear regression will not provide an accurate concentration of any sample with a concentration greater than 20µM. Out of 190 samples analysed for SRP in this investigation, only 5 were estimated to have a concentration >20µM, and these were all from the Iford site. For these 5 samples, a different standard curve was used (Figure 3.10). This provided a satisfactory solution to the problem of non-linearity in high concentration samples.

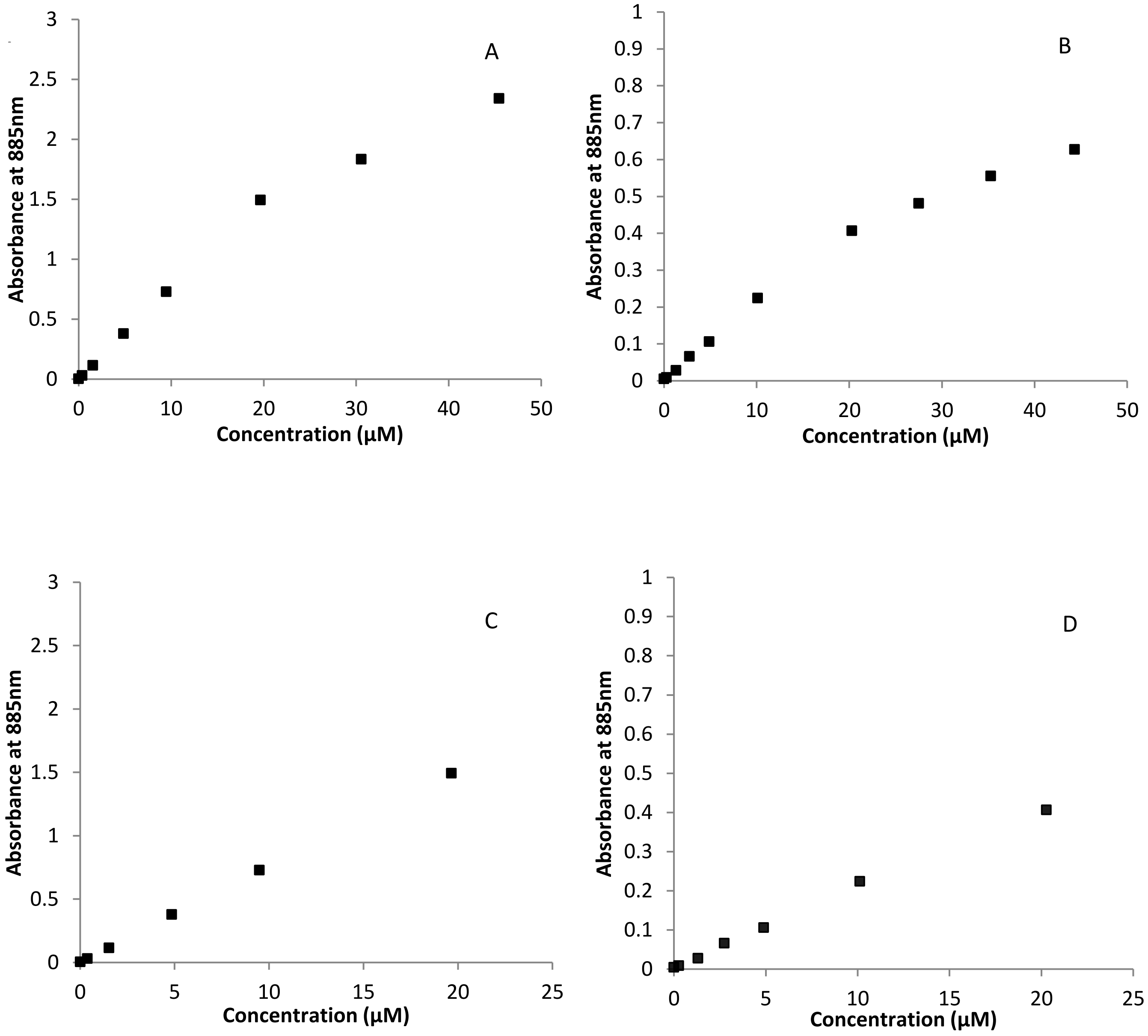


Figure 3.10: SRP standard curves using a 4cm pathlength cuvette (A and C) and 1cm pathlength cuvettes (B and D) A and B show the entire standard curve up to 45µM PO_4, C and D show the standard curve up to 20µM PO_4. Standards were analysed as single replicates.

3.4.4 Chemical oxidation of organic phosphorus

Sampling at Knapp Mill, Throop and Mudeford Quay commenced on the 16/4/13 and was implemented on a weekly basis until the 7/5/14. During this period, water samples were analysed for SRP within 24 hours of filtration, as described above and for TDP by potassium persulfate chemical oxidation assay. The method used in this study was an acidic potassium persulphate with autoclaving assay. 0.075g potassium peroxydisulphate powder was added to the sample along with 0.5ml 1N sulphuric acid. The samples were then autoclaved at 121°C for 45 minutes. After cooling, the samples were analysed for inorganic phosphate using the colourimetric SRP method described above. This was done with the aim of determining the organic phosphorus concentration by difference between the SRP detected after potassium persulphate digestion assay and SRP analyses of a non-digested sample. However, a consistent positive difference between the two analyses could not be reliably identified (Figure 3.11), and the SRP concentration was often higher than the TDP concentration. Because a suitable CRM was not available at this time to test the chemical digestion method further, an alternative method of analysing TDP was investigated.

Analysis of samples for TDP using the chemical oxidation method was stopped after 12/12/13 due to the lack of a consistent organic phosphorus signal. This may be to a number of issues in the analytical stage, such as contamination of the samples or contamination of the phosphate standard during filtration or sample preparation stage of the analysis. Another factor may be mistakes in the methodology which were not identified until the analysis was revisited at a late date. Despite the lack of an identifiable organic phosphorus signal, the results of this investigation are still important because it highlights the need for a robust alternative method to chemical oxidation, in addition to establishing the SRP analysis to be accurate and reproducible.

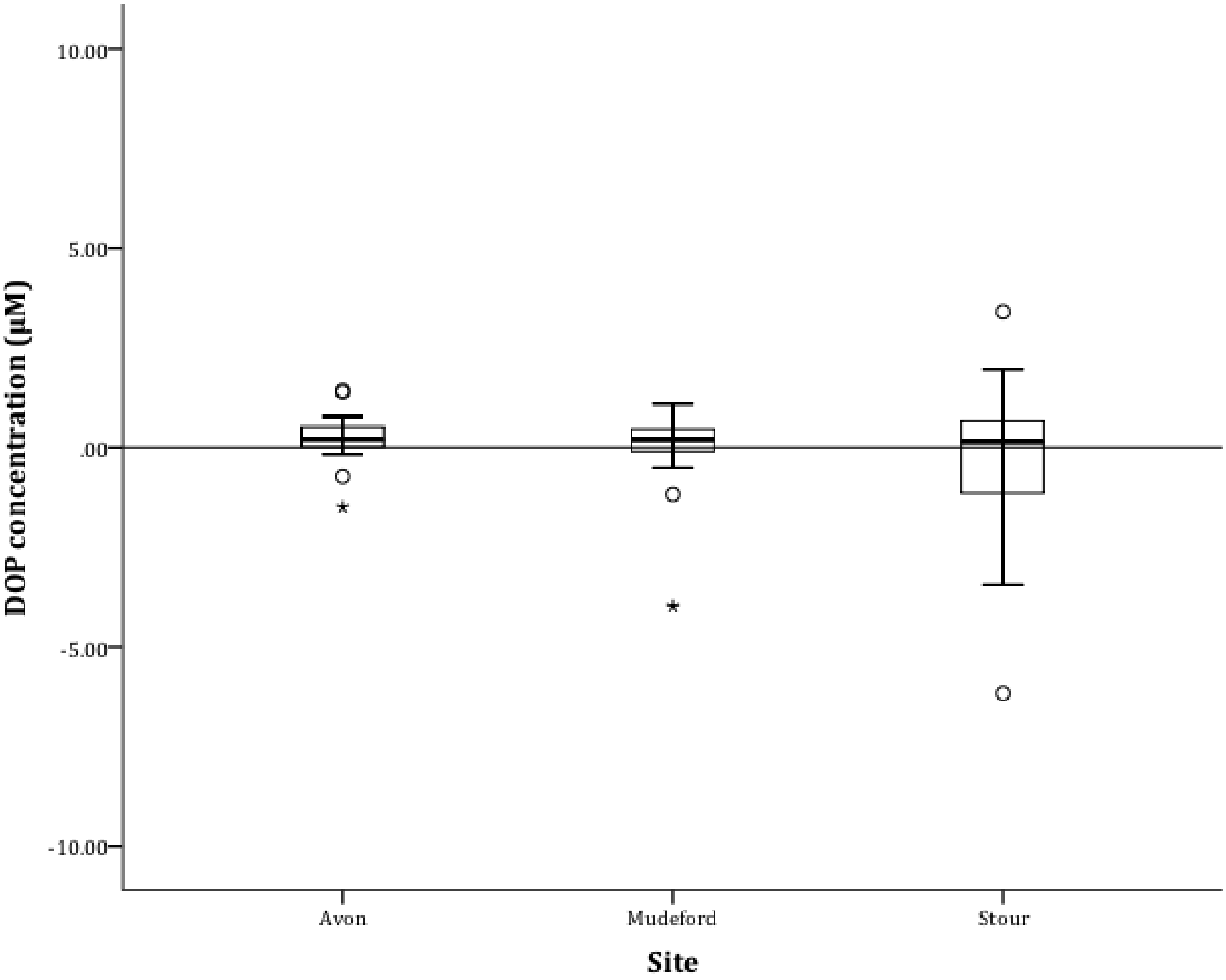


Figure 3.11: DOP concentration determined by subtraction of manually analysed SRP from TDP derived from chemical oxidation. Weekly collected samples were analysed from 24 dates from 4/7/13 to 9/12/13 (o and * show outliers).

3.4.5 Comparison of manual SRP to automated SRP analysis

A comparison was made between the SRP concentrations determined using a batch manual method in the labs at the National Oceanography Centre, Southampton (NOCS) on filtered frozen samples, with those preserved with Mercuric Chloride (unfrozen) and analysed by an autoanalyser at The University of Portsmouth. Both analyses were performed on water samples collected on the same day and returned to the lab in NOCS where they were passed through a pre-combusted 47mm Whatman GF/F filter (0.7µm pore size) prior to either freezing in PPE bottles or preserving with Mercuric Chloride in 20 ml HDPE plastic vials. Both methods of preservation of water samples prior to SRP analysis have been used in numerous previous studies (Kotlash and Chessman, 1998; Kattner, 1999, Lomas et al., 2010). A comparison of the concentration of SRP determined by the two independent assays on the same water sample from each of the sampling stations is shown in Figure 3.12.

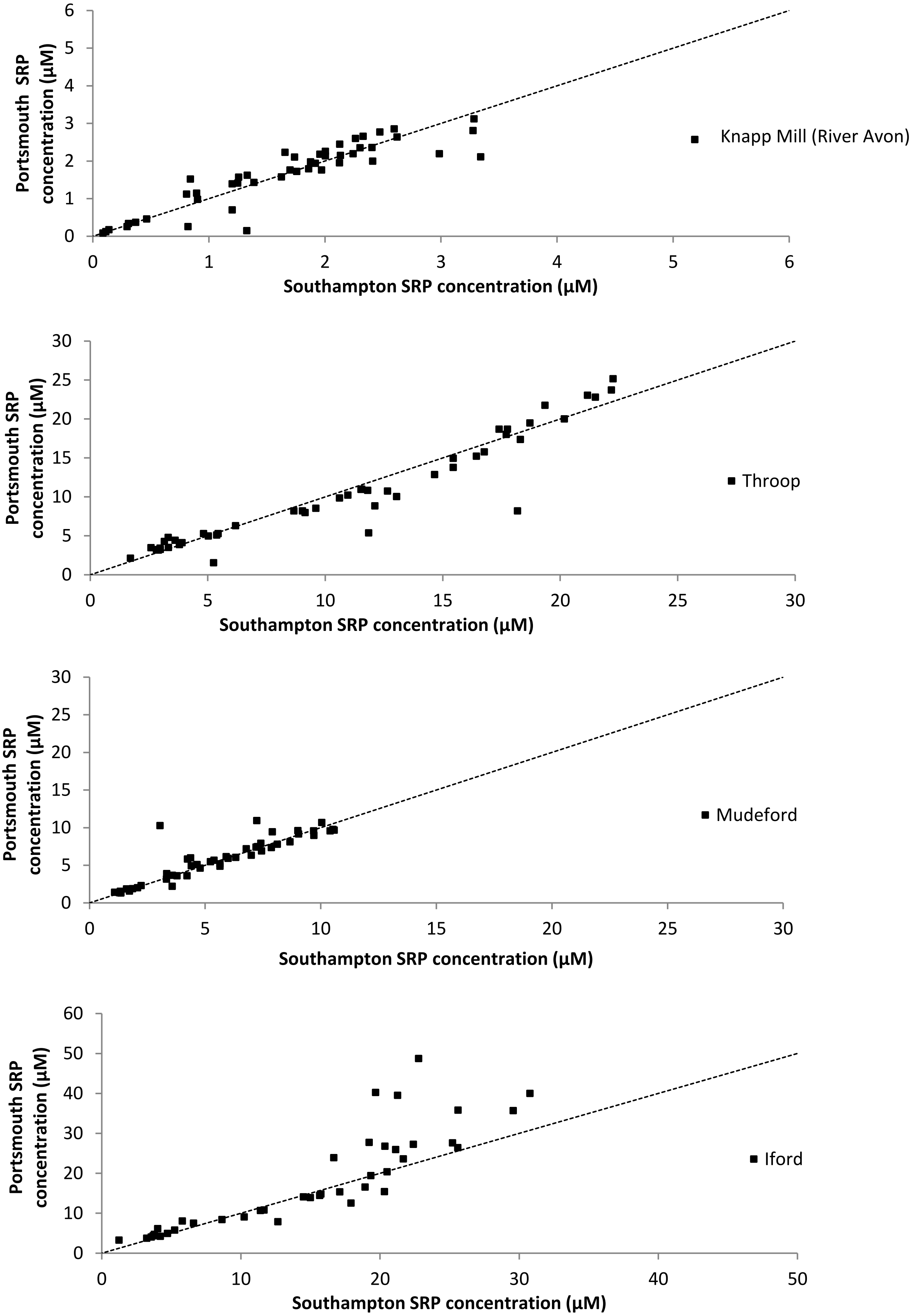


Figure 3.12: SRP concentration comparison between analysis done at the University of Portsmouth on an Autoanalyser and the manual SRP analysis from this investigation. Line = 1:1

The two sets of analyses show a close comparison of concentrations of SRP in brackish water samples collected from Mudeford Quay with little scatter around the 1:1 line (Figure 3.12). The samples collected at Throop and Knapp Mill show some scatter around the 1:1 line with samples from Throop having a much higher SRP concentration range. At both sites, the manual analyses yielded some lower concentrations of SRP compared to the autoanalyser analysed samples from the University of Portsmouth. Samples collected from Iford show a close comparison at concentrations below 20µM SRP however, above 20µM, the values derived from autoanalyser analysis yielded higher concentrations of SRP than the analysis done manually.

This comparison indicates that for Knapp Mill, Throop and Mudeford Quay, the majority of SRP samples analysed by both methods show close agreement but at concentrations above 20µM at Throop and Iford there is a discrepancy between the two analyses. As a certified reference material (CRM) for SRP was not available, this analysis does not definitively confirm the accuracy of the SRP analysis for either of the two methods. Nevertheless, because both sets of analysis were analysed independently, the similarity of the majority of SRP concentrations provides confidence in the SRP concentrations that are used to derive the DOP concentrations from the TDP concentrations, by difference.

3.5 Test analysis of organic Phosphorus using ICP-MS and spectrophotometry

The previously described experiments helped establish the optimal conditions and procedures for the analysis of organic phosphorus using a combination of ICP-MS for analysis of TDP and colourimetric analysis for SRP, with the ultimate aim of analysing a large number of stored environmental water samples. The complete results from these analyses are described in

Chapter 4. However, before this large pool of samples was analysed, a series of four test samples were analysed to establish whether the technique was applicable to the stored water samples. Samples from 4 different weeks of sampling at Knapp Mill on the River Avon were defrosted and analysed using the two techniques (Figure 3.13). A consistent distinct positive difference can be observed between the ICP-MS-derived TDP concentration and the colourimetric derived SRP concentration in all four samples indicating that the combined analysis could be extended for analysis of all the stored samples.

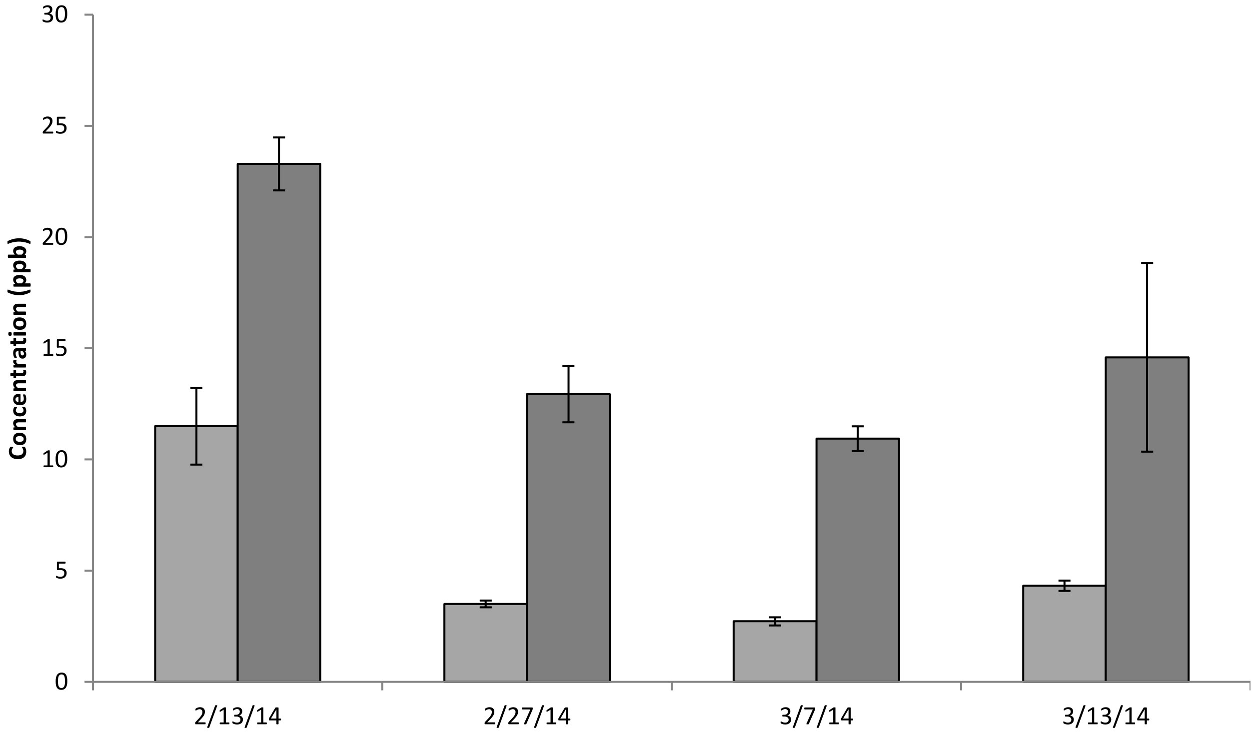


Figure 3.13: SRP (light grey) and TDP concentrations (dark grey) derived from and spectrophotometric analysis at 885nm and HR-ICP-MS analysis of 4 samples from the River Avon. Error bars show +/- 1sd. Each sample was replicated 3 times.

3.6 Discussion

A major drawback in the reporting of dissolved phosphorus in published research is a lack of a standardised method of analysis of total phosphorus (Worsfold et al., 2005). The differences in the efficiency of oxidation by various techniques adds an uncertainty to any TDP concentrations measured in this way (Ormaza-Gonzalez and Statham, 1996). This investigation uses a combination of HR-ICP-MS and spectrophotometry to measure TDP and SRP concentration respectively. The use of ICP-MS to analyse TDP has only been used in a small number of investigations (Cantarero et al., 2002, Ivanov et al., 2010, Regelink et al., 2013) and not on a dataset as large as the one used in this study. It is important to understand DOP dynamics because DOP has been observed to decrease the SRP demand of the phytoplankton community and is an important source of phosphorus for autotrophs (Dyhrman and Ruttenberg, 2006, Nausch and Nausch, 2007, Rofner et al., 2016).

Test analyses of organic phosphorus standards indicated that HR-ICP-MS was capable of analysing specific organic phosphorus compounds when prepared in a laboratory setting. In addition, there was a consistent difference (figure 3.13) between the SRP and the TDP concentration in four environmental water samples, a difference that can be operationally defined as dissolved organic phosphorus (DOP). Filtration was shown to decrease the total phosphorus (TP) concentration when analysed using ICP-MS. Filtration is a necessary step in the analysis of environmental water samples in order to reduce biological activity from phytoplankton and bacteria and phosphorus adsorption/release from particles (Jarvie et al., 2002; Lambert et al., 1992). Future analysis of phosphorus using ICP-MS to analyse the retentate of the filters used to filter the water samples would provide a particulate phosphorus (PP) concentration in conjunction with a TDP concentration, a factor which may be important to consider when investigating the partitioning of phosphorus in river and estuary systems. In addition to this, other methods of size fractionation such as ultrafiltration (Cai and Guo, 2009;

Majed et al., 2012) or field flow fractionation (Stolpe et al., 2010; Regelink et al., 2013) can be used prior to ICP-MS analysis to provide information on colloidal phosphorus.

A decrease in the linearity of the inorganic phosphate standard curve was revealed when the P concentration of the sample is above 25µM (Figure 3.10). This will not be a problem in detecting low concentration of P in oceanic samples but is more likely to cause problems in river water samples, particularly in water samples collected from river catchments which have a higher proportion of urban areas. The most suitable solution to this is prior digestion of the samples to a concentration below 25µM before spectrophotometric analysis.

No prior digestion of the water sample was needed to analyse TDP using ICP-MS (figure 3.7). This is another indicator that the argon plasma successfully ionizes the organic phosphorus molecules in the introduced water sample.

Freezing a sample was shown to reduce the phosphorus concentration, which is likely to be also due to biological activity or particle P release in the unfrozen sample (figure 3.5). This reinforces the need for a suitable storage procedure between collection of environmental water samples and analysis.

The SRP analysis used in this investigation was based on the technique described in Murphy and Riley, (1962). The standard curves created on when analysing batches of water samples on different days were reproducible and the concentrations of P in parallel samples analysed showed good agreement between the manual batch analysis conducted in laboratories in NOCS and the autoanalyser method conducted at The University of Portsmouth (figure 3.12).

The use of HR-ICP-MS to analyse phosphorus was demonstrated to provide fast and reliable concentrations of TDP and, when used in conjunction with the standard spectrophotometric analysis of SRP (Murphy and Riley, 1962), can provide an accurate DOP concentration in water samples. In addition, this investigation has shown that analysis of total phosphorus in filtered environmental water samples can be achieved quickly and accurately using ICP-MS without the use of a collision cell. One major advantage to this is the capability to combine ICP-MS with techniques such as high performance liquid chromatography (HPLC). HPLC has the capability to fractionate organic compounds prior to analysis with HR-ICP-MS (Heerboth, 2007; Atlas et al., 2015). However, a large number of phosphorus compounds do not adsorb in the visible or ultraviolet region and therefore analyses may require post-HPLC derivatisation for detection and quantification (Baldwin, 2013). Research into HPLC coupled to HR-ICP-MS is scarce at the current time but development of a technique to analyse total P in HPLC eluent would significantly improve analysis of environmental phosphorus in the future. Another advantage of the use of HR-ICP-MS to analyse TDP is the capability of multi-elemental analysis in conjunction with phosphorus. Any elements of interest can be analysed at the same time as phosphorus with only slight changes to the ICP-MS operating parameters.

3.6.1 Conclusions

This study has determined that HR-ICP-MS can be used to quickly and accurately measure TDP in environmental river water samples. This technique negates the need for any oxidising step prior to analysis and enables large batches of samples to be analysed at one time. The technique can be combined with a SRP assay to provide a DOP concentration in water samples, which can be a useful tool in understand phosphorus speciation and bioavailability in environmental systems.

Chapter 4: Dissolved organic phosphorus dynamics in the Christchurch Harbour system.

4.1 Introduction

Phosphorus is a macronutrient that is required for primary production and can be limiting in aquatic systems (Benson et al., 1996; Karl and Bjorkman, 2001). In natural waters, it exists in several chemical forms that may be categorised as either inorganic (protonated forms of PO_4^{3-}) or organic (Statham, 2012). These organic forms are of biological origin when formed naturally and include nucleic acids, polyphosphates, phosphorus esters and phosphonates (Clark et al., 1998; Benitez-Nelson, 2000; Kolowith et al., 2001), although man-made organic phosphorus compounds may also be present in polluted ecosystems. Dissolved organic phosphorus (DOP) is available to both phytoplankton and bacteria for production (Ormaza-Gonzalez and Statham, 1996; Huang and Hong, 1999; Kolowith et al., 2001) and can be transformed into inorganic phosphorus, leading to changes in the inorganic N/P ratio and potentially a shift in the planktonic community of a water body (Rinker and Powell, 2006).

DOP in aquatic and marine environments is a diverse assemblage of compounds including nucleic acids, other nucleotides (e.g. ATP, GTP and AMP), inositol phosphates, phospholipids and phosphonates (Baldwin, 2013). Esters are readily hydrolysed by bacterial and phytoplankton alkaline phosphatases (Chrost and Overbeck, 1987), and increases in phytoplankton phosphatase activity have been used as an indicator of SRP stress (Hoppe, 2003; Dyhrman and Ruttenberg, 2006). DOP has been observed to decrease the SRP demand of the phytoplankton community and is an important source of phosphorus for autotrophs (Dyhrman and Ruttenberg, 2006, Nausch and Nausch, 2007, Rotner et al., 2016), particularly in oligotrophic oceanic environments (Lomas et al., 2010, Dyhrman et al, 2006, Sebastián et al., 2012). In addition to this, in aquatic environments enriched in iron (oxyhydr)oxides, DOP bioavailability may exceed that of orthophosphate due to different sorption rates of SRP and DOP (Ruttenberg and Sulak, 2011). DOP can also account for over 80% of the TDP pool in the North Atlantic Ocean (Wu et al., 2000; Ammerman et al., 2003; Lomas et al., 2010) and has been recorded at over 75% of the total TDP pool in rivers (Neal et al., 2000a). Despite the increasing evidence for the importance of DOP utilization in marine and aquatic environments, relatively little is known about the seasonal variability in DOP concentrations in river and estuary systems and DOP concentrations in lowland river and estuary systems are not often measured. Monbet et al., 2009 analysed 8 stations from riverine to marine end-members on the River Tamar in Devon UK during four seasonal campaigns. DOP was found to comprise 6-40% of the TDP with the highest and lowest concentrations observed in autumn and summer, respectively. In addition to this, the total enzymatically hydrolysable phosphorus (EHP) pool, which consists of the whole DOP fraction hydrolyzed by the combination of three enzymes, was recorded. This labile portion of the total DOP constituted 6-40% of the DOP and behaved non-conservatively along a salinity gradient in the Tamar Estuary.

This chapter describes the seasonal changes in DOP concentrations and dynamics in the Christchurch Harbour estuarine system between April 2013 and April 2014. A combination of inductively coupled plasma mass spectroscopy (ICP-MS) analysis of total dissolved phosphorus (TDP) and colourimetric assay of soluble reactive phosphorus (SRP) as described by Murphy and Riley, (1962) was applied, as described in detail in chapter 3. This combination of analytical techniques allowed a distinct DOP fraction to be determined in the analysed samples.

The main aim of this chapter is to describe the changes TDP, SRP and DOP concentrations in the Christchurch Harbour estuarine system (Figure 1.3) and examine the relationships between them and other measured physiochemical and physical parameters. To the author’s knowledge, this is the first investigation to utilise ICP-MS to analyse TDP concentration in estuarine water samples. Concentrations of SRP will be compared to EU standards of SRP set by the EU Water Framework Directive, and the UK Technical advisory group on the Water Framework Directive (UKTAG, 2013). These standards were set in order to reduce nutrient delivery to river basins, estuaries and coastal margins within the EU.

In addition to the year's duration of sampling at each of the four sites, water samples were also collected from several sites within the Christchurch Harbour estuary during 5 transects conducted in the summer of 2014 and were subsequently analysed for the different phosphate fractions described above.

4.2 Materials and Methods

The collected water samples were subjected to a combination of ICP-MS analysis for TDP and the colourimetric inorganic phosphate analysis for SRP described in chapter 3. Briefly, samples were collected on a weekly basis between 16/4/13 to 8/4/14 from 4 sites; Throop and Iford on the River Stour, Knapp Mill on the River Avon and Mudeford Quay at the entrance to Christchurch Harbour estuary (Figure 4.1). Samples were filtered through a Whatman GF/F filter (pore size approximately 0.7µm) on return to the lab before freezing in 60ml HDPE bottles.

Analytical methods are described in detail in chapter 3. Briefly, ICP-MS analysis of TDP was performed on an Element 2 XR high resolution ICP-MS (Thermo Scientific, Germany). SRP analysis was performed using a colourimetric assay with colour development measured spectrophotometrically using a UNICAM 8625 spectrophotometer at 885nm. The subtraction of the SRP concentration from the TDP concentration gives the DOP concentration. All collected water samples were analysed in triplicate both for TDP and SRP.

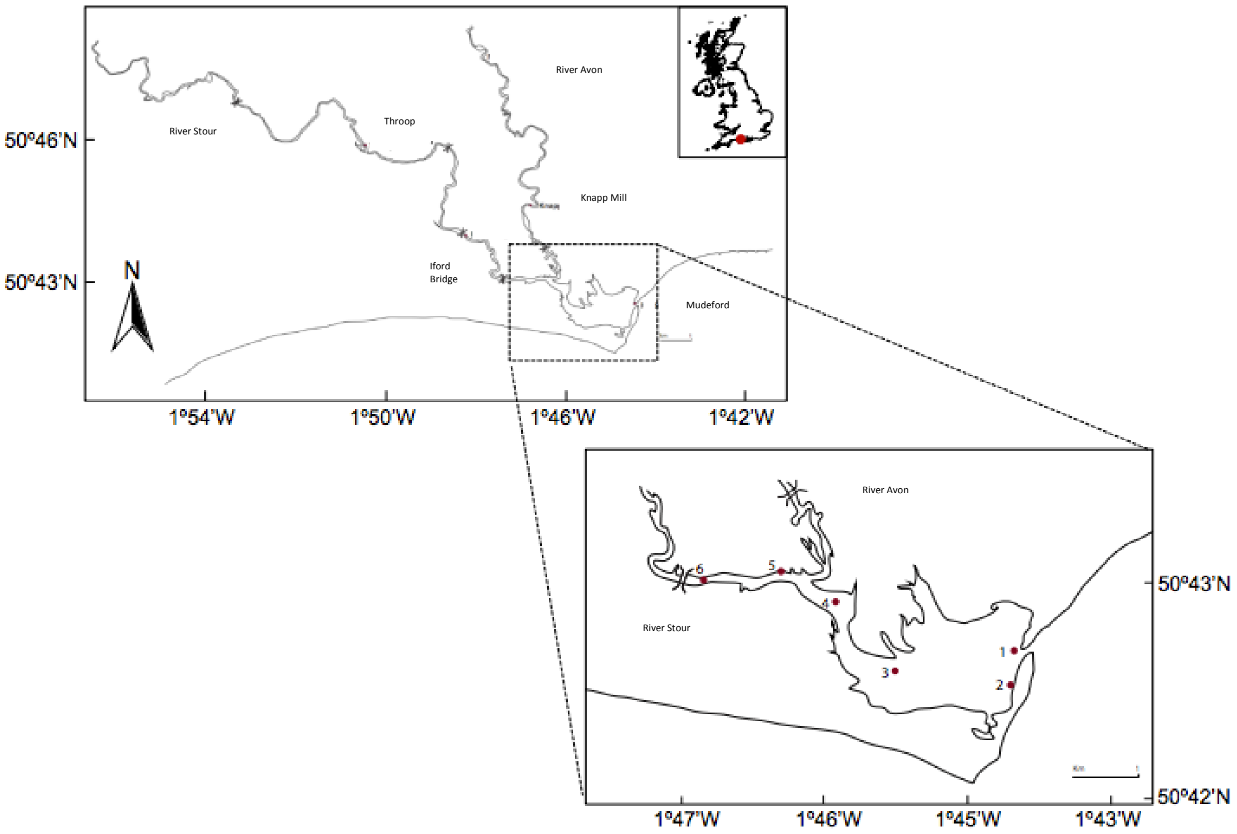


Figure 4.1: Location of the riverine and estuarine transect sites. Site 1 is Mudeford Quay, 2 is the Ferry Pontoon, 3 is Blackberry Point, is Grimbury Marsh, 5 is Christchurch Quay, 6 is Tuckton Bridge

4.2.1 Estuarine Transect sampling

Estuarine transect sampling was undertaken every 2 weeks from June to September at 6 sites (Figure 4.1) throughout the Christchurch Harbour estuary on, 12/6/14, 25/6/14, 10/7/14, 24/7/4 and 21/8/14). A YSI 6600 multiprobe sonde was deployed and vertical measurements of temperature, salinity/conductivity, chlorophyll a concentration, oxygen saturation and turbidity were taken at 0.5m intervals throughout the water column. In addition to this, water samples were collected using a Niskin bottle for later TDP and SRP analysis for 5 of these transects.

Water samples were collected from a depth close to the chlorophyll maximum as determined from the YSI EXO 2 multiprobe profile. The water was collected in 1L HDPE bottles and stored in a cool box before transit to the laboratory. The water was then filtered through a Whatman GF/F filter (0.7µM) and frozen for later TDP and SRP analysis.

4.3 Results

4.3.1 Total dissolved phosphorus (TDP)

Figure 4.2 shows the TDP concentration measured in water samples collected from the four sites on a weekly basis between 16/04/2013 and 8/04/2014. The TDP in the River Avon at Knapp Mill ranged between 0.4µM on the 7/3/14 and 3.8µM on the 9/12/13 (Table 4.1, Figure 4.2). The TDP in the River Stour at Throop ranged from 28.4µM on the 11/10/13 to 2.6µM on the 3/5/13. At Iford the TDP concentration ranged from 3.4µM on the 13/3/14 and 43.8µM on 16/10/13 and at Mudeford, the minimum TDP concentration was 1.06µM on the 6/7/13 and the maximum was 28.2µM on the 6/8/13. The maximum, minimum and range of the TDP concentration at Knapp Mill on the River Avon was considerably lower than the other three sites. The TDP concentration from April to July 2013 increased from around 2µM to 3.5µM in August. This was followed by a decrease back to approximately 2µM in September before increasing to the maximum of 3.8µM in December. During high river flow periods, the TDP concentration decreased to its lowest concentration in January. The TDP concentration remained low until the flow started to decrease when it then returned to 2µM in April 2014.

A similar seasonal pattern was observed at Throop; however, the overall concentrations of TDP at Throop were consistently higher than at Knapp Mill (Figure 4.2). The concentration decreased from 6.9µM on 26/4/13 to 1.7µM on 3/5/13. The TDP concentration then increased steadily to a maximum of 22.2µM on 25/9/13. The concentration then decreased sharply to 9.2µM on 30/10/13, coinciding with an increase in the river flow. The TDP concentration remained at approximately this level until a large spike on 16/12/13, coinciding with the start of the high rainfall and high river flow event in late 2013-early 2014. The concentration then dropped to between 3.3µM and 5.6µM until 19/3/14 when the TDP started to rise until the final analysis on the 10/4/13 where the TDP concentration was 7.3µM.

Table 4.1: Maximum, minimum, mean and standard deviation of the SRP, TDP and DOP concentration at the four sites from 26/4/13 to 10/8/14. 50 samples were analysed at Knapp Mill, Throop and Mudeford and 40 at Iford. BDL= below detection limit (0.02µM).

		Knapp Mill	Throop	Iford	Mudeford
SRP	Max (µM)	3.3	22.3	30.8	17.0
	Min (µM)	0.1	1.71	1.3	1.0
	Mean (µM)	1.6	10.9	15.0	5.7
	Standard deviation	0.8	6.4	8.2	3.3
TDP	Max (µM)	3.8	28.4	43.8	28.20
	Min (µM)	0.4	2.6	3.4	1.1
	Mean (µM)	2.1	12.9	17.8	10.4
	Standard deviation	0.87	7.8	11.2	7.1
DOP	Max (µM)	1.5	12.1	21.0	17.8
	Min (µM)	BDL	BDL	BDL	BDL
	Mean (µM)	0.5	2.0	2.8	4.7
	Standard deviation	0.4	2.2	5.0	4.9
DOP %	Max	75.1	46.9	65.0	68.1
	Min	0.0	0.2	0.0	2.8
	Mean	27.4	15.5	14.4	37.9
	Standard deviation	20.6	9.8	15.9	22.0

The site at Mudeford Quay is tidal and so is affected both by river flow and changes in salinity from the seawater entering the estuary. The salinity varied from 0.21 to 22.5 in samples collected over the sampling period and may have influenced the TDP concentration. However, efforts were made throughout the sampling regime to sample the site at Mudeford at low tide to increase the proportion of river water to seawater in the samples. As for the other three sites, a seasonal signal can be observed at Mudeford Quay. The TDP concentration increased from 8.9µM on the 16/4/13 to a maximum of 28.2µM on 6/8/13. The concentration then decreased to 4.1µM on the 24/10/13. After this initial increase in TDP concentration, the TDP concentration was diluted by the large volume of river water and remained low, at approximately 5µM until the riverine input decreased in March 2014.

The TDP concentration at Iford was very variable; varying between 11.4µM and 40.6µM between 6/6/13 and 11/10/13 with a maximum-recorded TDP concentration of 43.8 on the 16/10/13 (Figure 4.2). After this, the TDP concentration dropped to 11.7µM on 7/10/13 before increasing to 22.0µM on 16/12/13. This was followed by a decrease in TDP concentration to between 1.3µM and 6.61µM between 23/1/14 and 27/3/14 coinciding with the large rainfall event. Notable at Iford is a lack of a large peak in TDP, which was evident at Throop on 16/12/13.

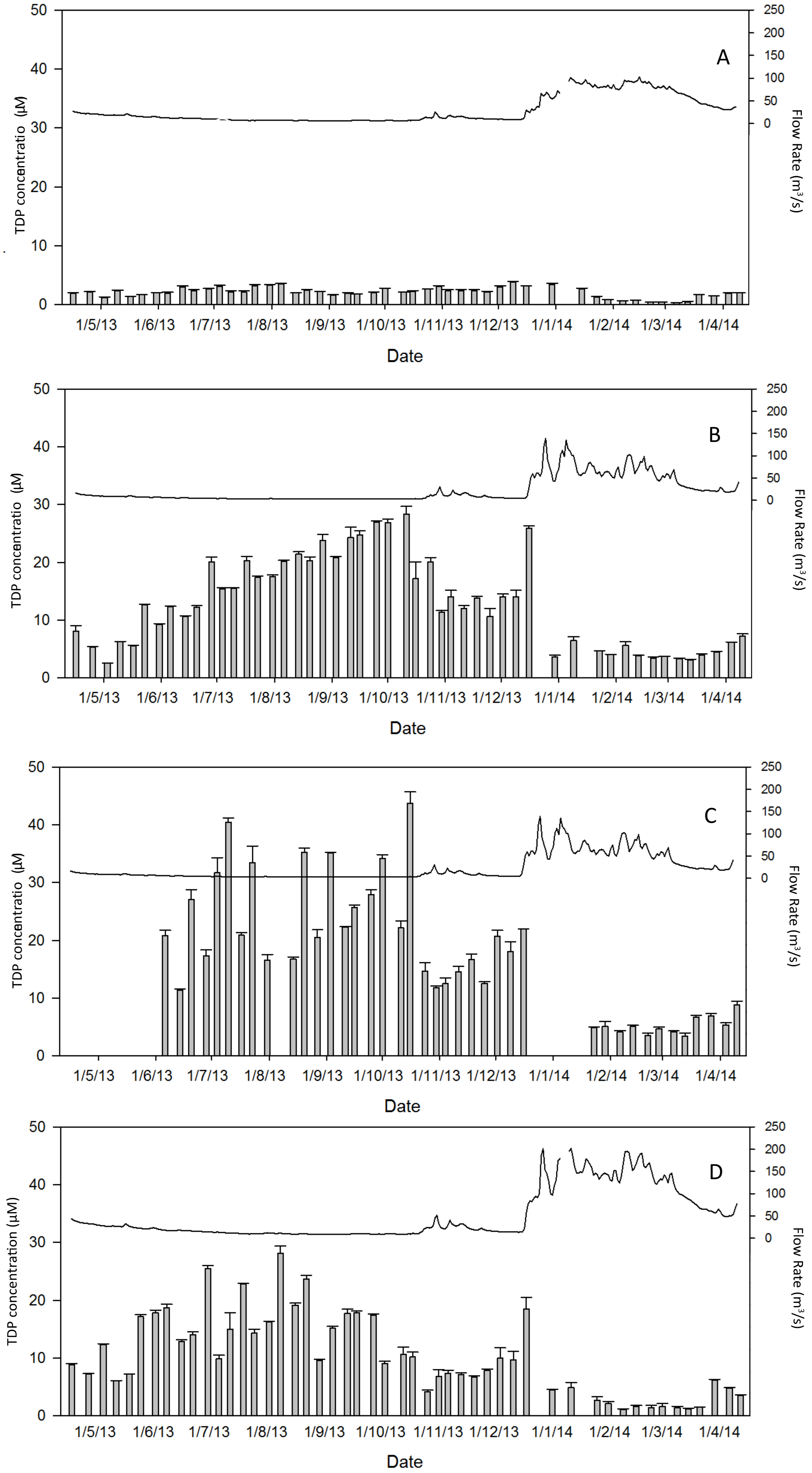


Figure 4.2: Total dissolved phosphorus (TDP) concentration at Knapp Mill (A), Throop (B), Iford (C) and Mudeford Quay (D). Error bars show ± 1 sd of 3 replicates. The line shows the daily mean river flow rate at each of the sites. The combined flow rate of the River Avon at Knapp Mill and the River Stour at Throop are used for Figure D.

4.3.2 Soluble Reactive Phosphorus (SRP)

The SRP concentration at Knapp Mill on the River Avon varied from 0.1µM on the 7/3/14 to 3.3µM on the 9/12/13 (Table 4.1, Figure 4.3). The SRP concentration remained stable from April to July at 1-2µM then increased through August and September to 2-3µM. The concentration was stable at approximately 2µM in November before rising to approximately 3µM in December. The concentration dropped to <0.5µM in January and remained low until March, coincident with high river flow rates.

SRP analysis of samples from Throop on the River Stour show concentrations of SRP in April 2013 of 6.9µM before dropping to 1.7µM on (3/5/13). The SRP concentration increased steadily during the summer months with a maximum SRP concentration of 22.3µM on (25/9/13). The concentration of SRP then dropped to 9.2µM on the 30/10/13 before increasing to 13.7µM on the 16/12/13. The SRP concentration then dropped to 3.3µM with increased river flow. The SRP concentration then remained at approximately 2-3µM until the flow rate decreased in March 2014 and the SRP concentration returned to approximately 6µM.

Mudeford Quay is a tidal site at the mouth of Christchurch Harbour so is affected by saline intrusion of seawater, particularly at high tide or during periods of reduced flow from the rivers. The concentration of SRP on 16/4/13 was 3.3µM. The SRP concentration then rose to 9.0µM on the 31/5/13. Between 31/5/13 and3/9/13 the SRP concentration varied between 10.6µM and 4.2µM on an almost fortnightly cycle. The SRP concentration peaked on the 16/12/13 then decreased to approximately 2µM until 19/3/13 when the SRP concentration increases back to approximately 3µM.

The site at Iford Bridge on the River Stour is 3.6km downstream from Throop and 2.2km downstream from Holdenhurst sewage treatment works. Sampling at this site only started on 6/6/13. The SRP concentration varied between 14.5µM and 30.7µM between 6/6/13 and 16/10/13 (Figure 4.3). The SRP concentration then decreased to 10.3µM on the 30/10/13 coinciding with a small peak in river flow. The SRP concentration then rose to 20.5µM on the 2/12/13 with decreasing river flow. The SRP concentration then decreased sharply to approximately 3µM for the duration of the high flow period in early 2014. As the river flow from this event decreased, the SRP concentration at Iford rose to 8.7µM on 10/4/13, the last sampling point.

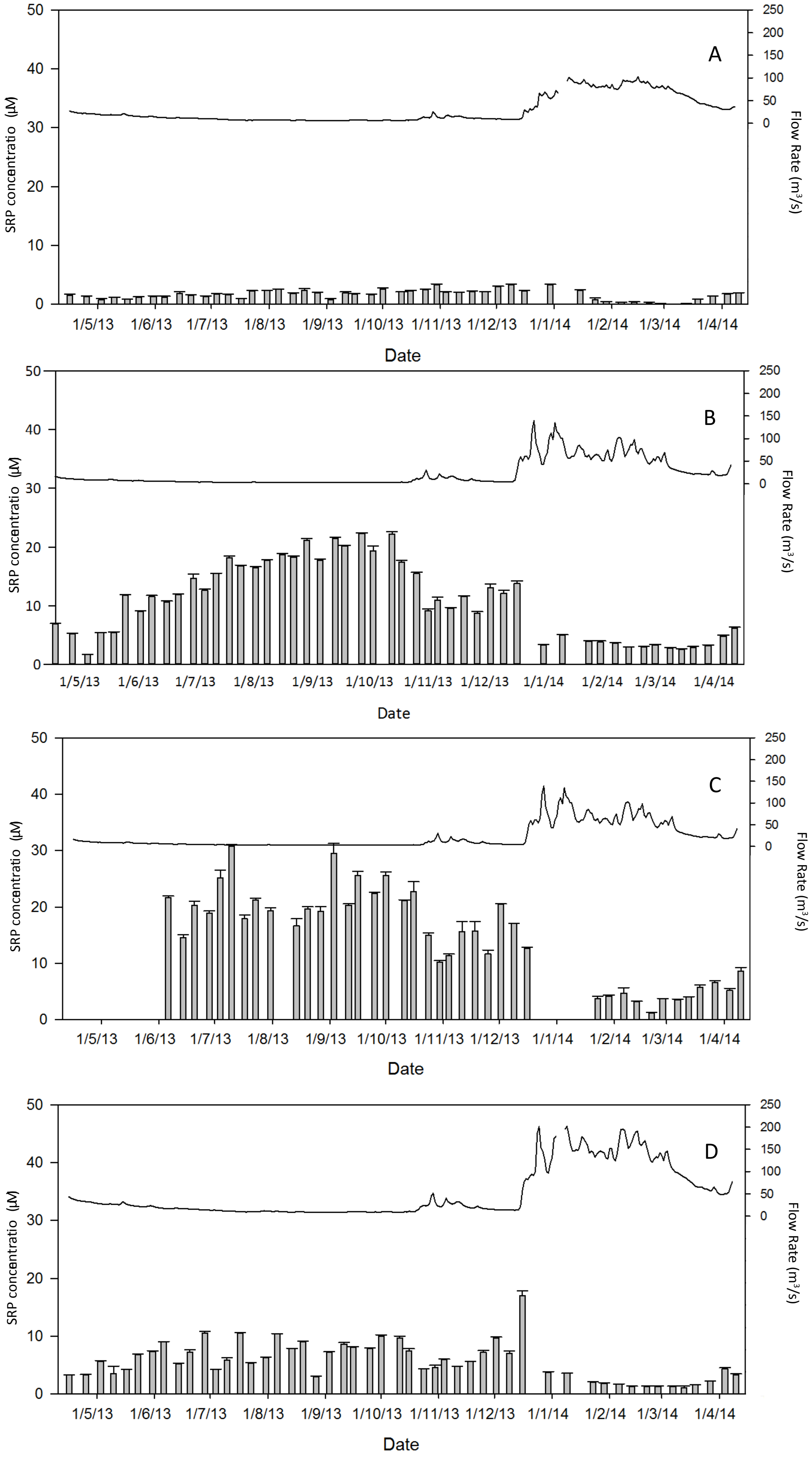


Figure 4.3: Soluble reactive phosphorus (SRP) concentration at Knapp Mill (A), Throop (B), Iford (C) and Mudeford Quay (D). Error bars show ± 1 sd of 3 replicates. The line shows the daily mean river flow rate at each of the sites. The combined flow rate of the River Avon at Knapp Mill and the River Stour at Throop are used for Figure D.

4.3.3 Dissolved organic phosphorus (DOP)

The DOP concentration was obtained by subtracting the SRP concentration from the TDP concentration measured in each filtered water sample. The DOP concentration of the River Avon at Knapp Mill ranged from 1.5µM on the 4/7/13 to undetectable (detection limits for DOP were 0.02µM with a coefficient of variation of 1%) (Figure 4.4). A concentration of 0µM DOP was therefore assumed on a number of dates, indicating that any DOP present was below detection limits using a combination of ICP-MS for detecting TDP and the colourimetric method of analysing SRP. The DOP concentration was highest in June and July, with an average DOP concentration in these months of mostly 1.0µM – 2.0µM. After June and July, the DOP concentration at Knapp Mill decreased to less than 0.4µM until December with the exception of 27/8/13. There was a peak in DOP concentration in December, corresponding with an increase in flow rate. The DOP concentration then decreased after December 2013 to approximately 0.4µM.

The DOP concentration at Throop on the River Stour ranged from 12.1µM on the 16/12/13 to undetectable concentrations on a number of occasions. From the 16/4/13 to the 6/6/13 the DOP concentration was between 1.2 µM and 0.1µM. There was a spiked increase in DOP to 5.5µM on the 20/6/13 before returning to the previous level on 4/7/13. The DOP concentration then increased steadily to 7.5µM on the 25/9/13 followed by a steady decrease to 1.0µM on the 25/11/13. There was a large spike in DOP concentration to 12.1µM on the 9/12/13, corresponding to an increase in flow rate. After this large apparent release of DOP, the DOP concentration decreased to approximately 0.2µM until April 2014.

The DOP concentration at Iford Bridge on the River Stour ranged from 21.0µM on the 11/10/13 to undetectable concentrations. The variability between weeks is high and large concentrations of DOP were detected throughout the summer months. However, from the 24/10/13 to 2/12/13 low DOP concentrations were measured. There is a spike in DOP concentration on the 9/12/13 to 9.3µM. The DOP concentration at Iford was then undetectable until 15/1/13 where the concentration of DOP was 1.2µM. The DOP concentration then remained low until April 2014.

At Mudeford Quay DOP concentrations ranged from 17.8µM on the 6/8/13 to undetectable concentrations. The DOP concentration was elevated for the period of 16/4/13 to 25/9/13. After this period, the DOP concentration at Mudeford remained below 3µM for the rest of the sampling period except for on the 19/3/14 when the DOP concentration increased to 4.0µM.

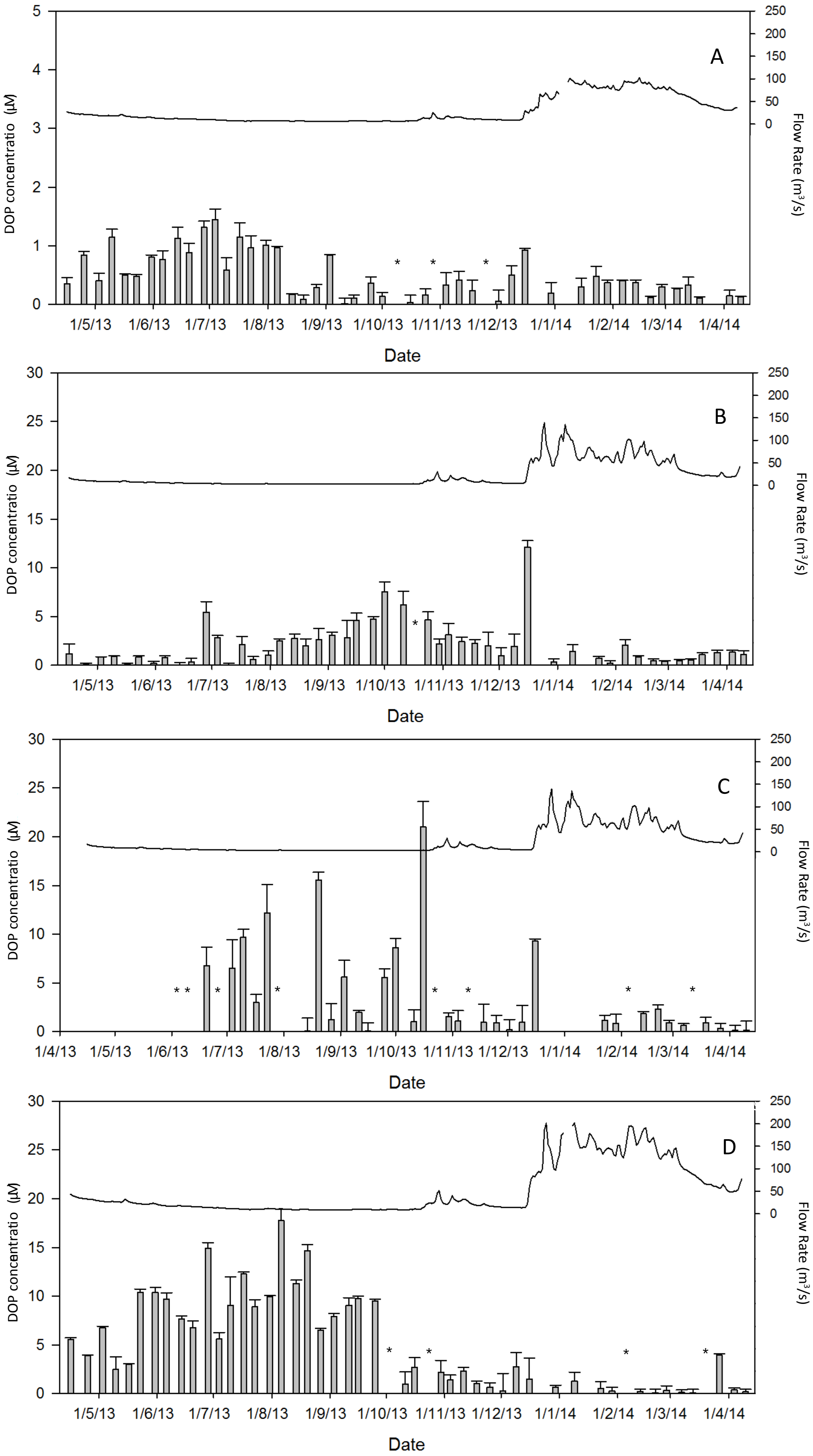


Figure 4.4: Dissolved organic phosphorus (DOP) concentration at Knapp Mill (A), Throop (B), Iford (C) and Mudeford Quay (D). Error bars show ± 1 sd of 3 replicates. Points where the DOP concentration was below detection limits are indicated by an *. The line shows the daily mean river flow rate at the sites. The combined flow rate at Knapp Mill and at Throop are used for Figure D.

The average SRP concentration at Throop was 10.9µM measured over the year compared to 1.6µM at Knapp Mill (Table 4.1) and the SRP concentration was higher at Throop than at Knapp Mill on every date on which SRP was analysed. There was also a notable seasonal pattern noticeable at Throop with the SRP concentration increasing over the summer to reach a peak on the 11/10/13 before decreasing in concentration. Compared to Iford, the SRP concentration at Throop was consistently lower with the exception of 5 sampling dates. However, the SRP concentration at Throop and Iford show similar seasonal patterns with a peak in September before a reduction in SRP concentration related to river flow rate increase. The site at Iford shows large peaks in SRP concentration compared with the 2 other riverine sites and peaks are evident at Mudeford Quay on the same dates but at lower concentrations. Samples from the River Avon at Knapp Mill consistently had the lowest SRP concentrations of the 4 sites. Particularly absent from Knapp Mill is the presence of a large peak in SRP concentration from May to October, which is evident at the other sites. All four of the sites exhibit a significant reduction in SRP concentration from January to April 2014 in relation to increased high flow rates of the rivers. The estuarine site at Mudeford is influenced by river water from the Stour and Avon, in addition to seawater and inputs from minor freshwater tributaries to the harbour such as the River Mude and Bure Brook. This is evident as the SRP concentration at Mudeford is consistently lower than at Throop and Iford but consistently higher than at Knapp Mill. Mudeford shows the large peaks in SRP seen at Iford but these peaks are lower, most likely due to the buffering effect of the low SRP water from the River Avon. Mudeford also exhibits low SRP concentrations in early 2014.

The TDP concentration in the River Avon was consistently the lowest of all the sites and there was no peak over the summer months, as seen at the other three sites. There was a reduction in early 2014, associated with a high river flow rate. However, unlike the other sites, there is no large peak in late December 2013 associated with high run-off from the land.

The TDP concentration at Throop is consistently higher than the TDP concentration at Knapp Mill. The TDP concentration at Throop is also much more variable that Knapp Mill. There is a large peak from 25/4/13 to 11/10/13 up to 28.4µM and then an evident peak on 16/12/13 associated with increased run off. The TDP concentration at Iford shows a similar seasonal pattern to Throop but has numerous spikes of high TDP detected throughout the period between 14/6/13 to 16/10/13. The TDP concentration at Mudeford is also elevated over the period from May to September. There are peaks in TDP on 28/6/13 and 17/7/13, which match up with peaks in TDP at Iford and Throop. The summer peak of TDP at Mudeford decreased earlier than at the sites on the River Stour, on the 20/8/13 as opposed to 11/10/13. The increase in TDP on 16/12/13 observed at Throop and Iford is also evident at Mudeford.

DOP concentration of water samples from Knapp Mill and Throop from 16/4/13 to 23/7/13 were both lower than 1.5µM with the exception of a spike in DOP concentration to 5.5µM on the 28/6/13 at Throop. However, the DOP concentration at Throop was elevated from the 23/7/13 until a peak at 7.5µM on 1/10/13. During this period, the concentration at Knapp Mill remained low. There is a large spike in DOP on the 16/12/13 at Throop not evident at Knapp Mill. Iford on the River Stour exhibited much more variable DOP concentrations than at Throop with spikes in DOP concentration on 14/6/13, 4/7/13, 17/7/13, 14/8/13 and 16/12/13. Between these spikes the concentration DOP at Iford returned to lower concentrations.

DOP is operationally defined as the difference between SRP and TDP. The relative proportions of these two fractions changes throughout the year and varies between sample sites. The River Avon at Knapp Mill had a maximum DOP proportion of 75.1% of the TDP on 27/2/14 and a mean DOP % of 27.4% (Table 4.1). Between 16/4/13 and 6/8/13 the DOP % at Knapp Mill varied between 18.3% and 53.6% (Figure 4.5). The DOP % decreased to 3.7% on 20/8/13. There was an increase to 49.9% on 3/9/13. The DOP % then decreased to between

1.0% and 19.0% from 11/9.13 to 2/12/13. The DOP % then increased again to 56.6% on 6/2/14 before a drop to 29.3% in 21/2/14. There was then another spike to 75.1% on 7/3/13. The DOP % then sharply dropped to between 0% and 8.0% for the remainder of the sampling period. The River Stour at Throop reached a maximum DOP proportion of 46.9% on 16/12/13 with an annual mean DOP % of 15.5. Between 16/4/13 and 23/7/13 the DOP % ranged between 14.8% and 0.5% with the exception of two spikes on 3/5/13 and 28/6/13 to 33.3% and 27.1% respectively. The DOP % then rose steadily to a peak of 28.0% on 1/10/13. The DOP % stayed at a relatively steady proportion until a dip followed by a peak to 46.9% on 16/12/13. This was followed by another dip before a peak to 36.13% on 6/2/13. The DOP % then decreased to 13.8% on 21/2/14 before a rise to 27.11 % on 19/3/14. The River Stour at Iford showed a very varied DOP % with a number of large peaks in DOP % on 23/7/13, 20/8/13, 16/10/13, 16/12/13 and 21/2/14 followed by a decrease in DOP % to almost 0. The site at Mudeford had two distinct phases of DOP % from 16/4/13 to 25/9/13 the DOP % varied from 41.1% to 68.2%. The DOP % then begins a second phase of lower DOP proportion from 11/10/13 to 10/4/13 the DOP % varied between 2.8% and 32.5% with the exception of a large spike on the 27/3/14 to 63.8%.

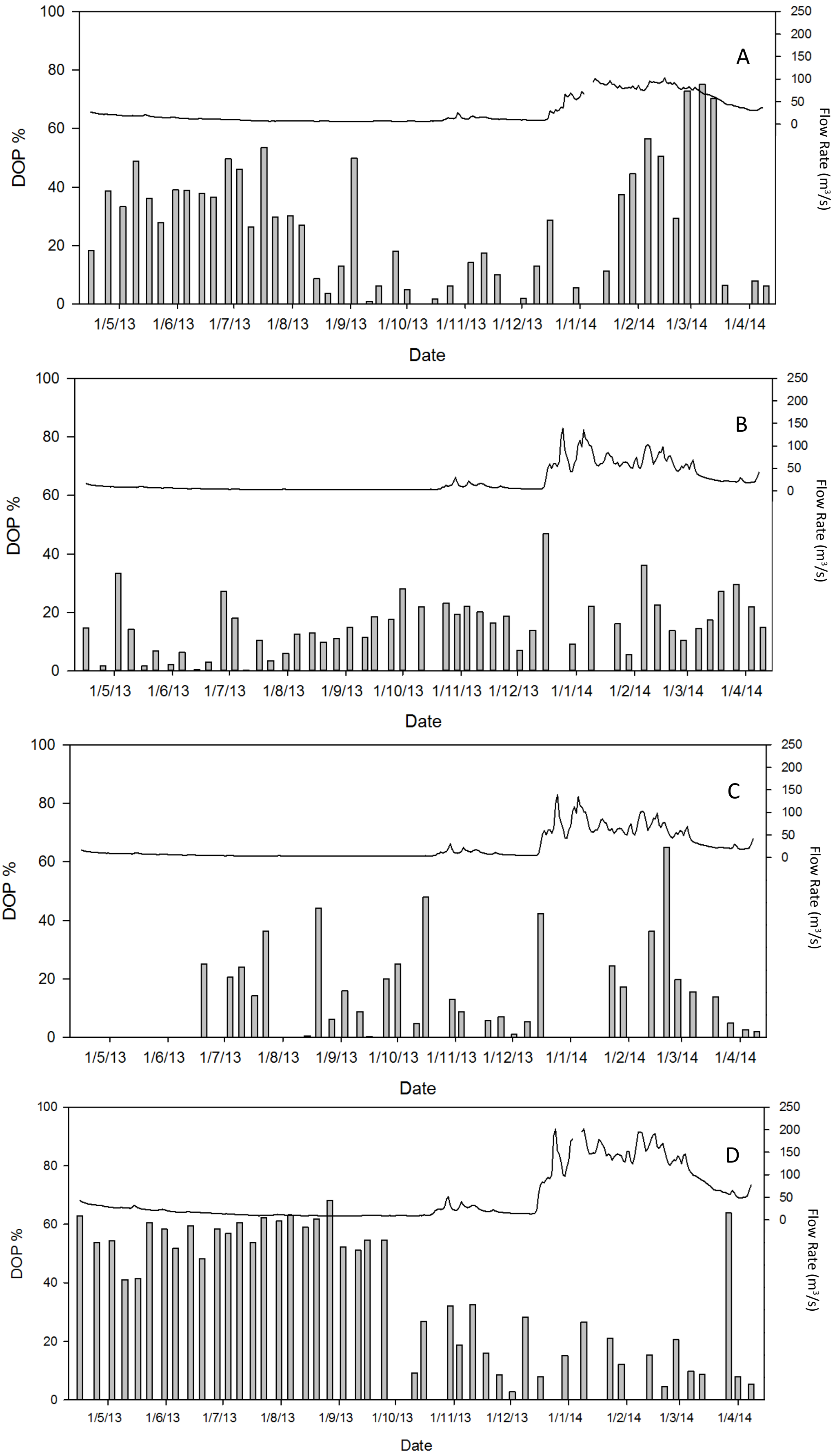


Figure 4.5: Dissolved organic phosphorus % (DOP%) at Knapp Mill (A), Throop (B), Iford (C) and Mudeford Quay (D). Error bars show ± 1 sd of 3 replicates. The line shows the daily mean river flow rate at each of the sites. The combined flow rate of the River Avon at Knapp Mill and the River Stour at Throop are used for Figure D.

4.3.4 Fluxes of Phosphorus

The absolute concentrations of TDP, SRP and DOP from discrete water samples collected on an almost weekly basis from April 2013 to April 2014 have been presented in the previous sections of this chapter. Nutrient fluxes for the river sites were calculated by multiplying the measured concentration of each nutrient by the daily mean flow for the same day at that site. For the estuary the daily mean flow for both rivers were summed together to determine the total daily mean flow and the nutrient concentrations measured at the estuary mouth were multiplied by this value (Figure 4.6). This is a useful measurement because it provides a picture of the relative contributions of each river to the P delivery into the Christchurch Harbour estuary. The flux at Iford can be estimated using the flow data from the site at Throop as there is only a small tributary that enters the River Stour between Throop and Iford, the River Moors, which has low average flow in comparison with the River Stour. The flow rate at Mudeford is calculated using the sum of the daily mean flow rate at Knapp Mill and the daily mean flow rate at Throop as a proxy to the total river flow entering the estuary.

The River Avon at Knapp Mill had a higher average flow rate (28.6m^3/s) than the River Stour at Throop (22.3m^3/s) over the year of sampling but in 29 of the 355 days in which it was recorded, the mean daily flow rate at Throop was higher than the mean daily flow rate at Knapp Mill. The maximum and minimum flow rate at Knapp Mill was 102.6m^3/on the 15/2/14 and 5.83m^3/s on 3/9/13 and at Throop the maximum flow was 139.54m^3/s on the 25/12/13 and the minimum was 2.43m^3/s on 11/10/13. The flow rate at Knapp Mill on the River Avon is higher the majority of the time, but as has previously been identified; the TDP, SRP and DOP concentrations are consistently higher at Throop. This results in the flux of P at Throop being significantly higher than at Knapp Mill. In addition to this, the TDP concentration at Throop has spikes in P on the 24/10/13, 16/12/13 and 6/2/14, which were not detected at Knapp Mill, where the flux of TDP remains relatively constant, despite the increase in river flow rate. The

SRP flux at Knapp Mill is consistently lower than at Throop from 16/4/13 to 11/10/13 during which period the fluxes at both sites remain fairly constant, with no spikes in the amount of SRP being transported down the rivers. However, there is a spike in SRP flux at Throop on the 24/10/13 coinciding with an increase in river flow. The SRP flux at Throop decreases back to the previous level on the 18/11/13. There is another large spike in SRP flux at Throop on the 16/12/13, again coinciding with an increase in river flow rate. This increase in SRP is not seen at Knapp Mill on the River Avon until the 30/12/13. The SRP flux at Knapp Mill returned to the previous level on 21/2/14 whereas at Throop the SRP flux does not return to the base level until 13/3/14. The DOP flux at Throop is very variable, with a maximum flux of 0.45 Kg DOP d^{-1} on 16/12/13 to undetectable fluxes. The DOP flux at Throop on 16/4/13 to 20/6/13 ranged between 0.05 Kg DOP d^{-1} and undetectable fluxes. There is a large spike the DOP flux on the 28/6/13 to 0.21 Kg DOP d^{-1}. The flux then dips to 0.05 Kg DOP d^{-1} on the 31/7/13 before rising to 0.39 Kg DOP d^{-1} on the 1/10/13. The DOP flux drops to 0.04 Kg DOP d^{-1} on the 2/12/13 before a large peak to 0.45 Kg DOP d^{-1} on 16/12/13. The DOP flux then decreases to undetectable amounts from January 2014 to April 2014 during periods of high river flow. The DOP flux in the river Avon ranged from negligible fluxes to 0.10 kg DOP d^{-1}. The DOP flux was lower at Knapp Mill than at Throop, with the exception of the high flow period in early 2014 where the DOP concentration at Knapp Mill was higher.

During flood events, the export of nutrients was higher than during low flow periods. From 16/12/13 to 27/3/14, the period of high flow, 14 samples were collected (total samples collected was 51). During this time period, 47.4% of the annual TDP flux, 45.2% of the annual SRP flux and 58.0% of the annual DOP flux was seen at Knapp Mill. At Throop, 53.4% of the annual TDP flux, 49.54% of the annual SRP flux and 13.4% of the annual DOP flux was observed during the high flow period.

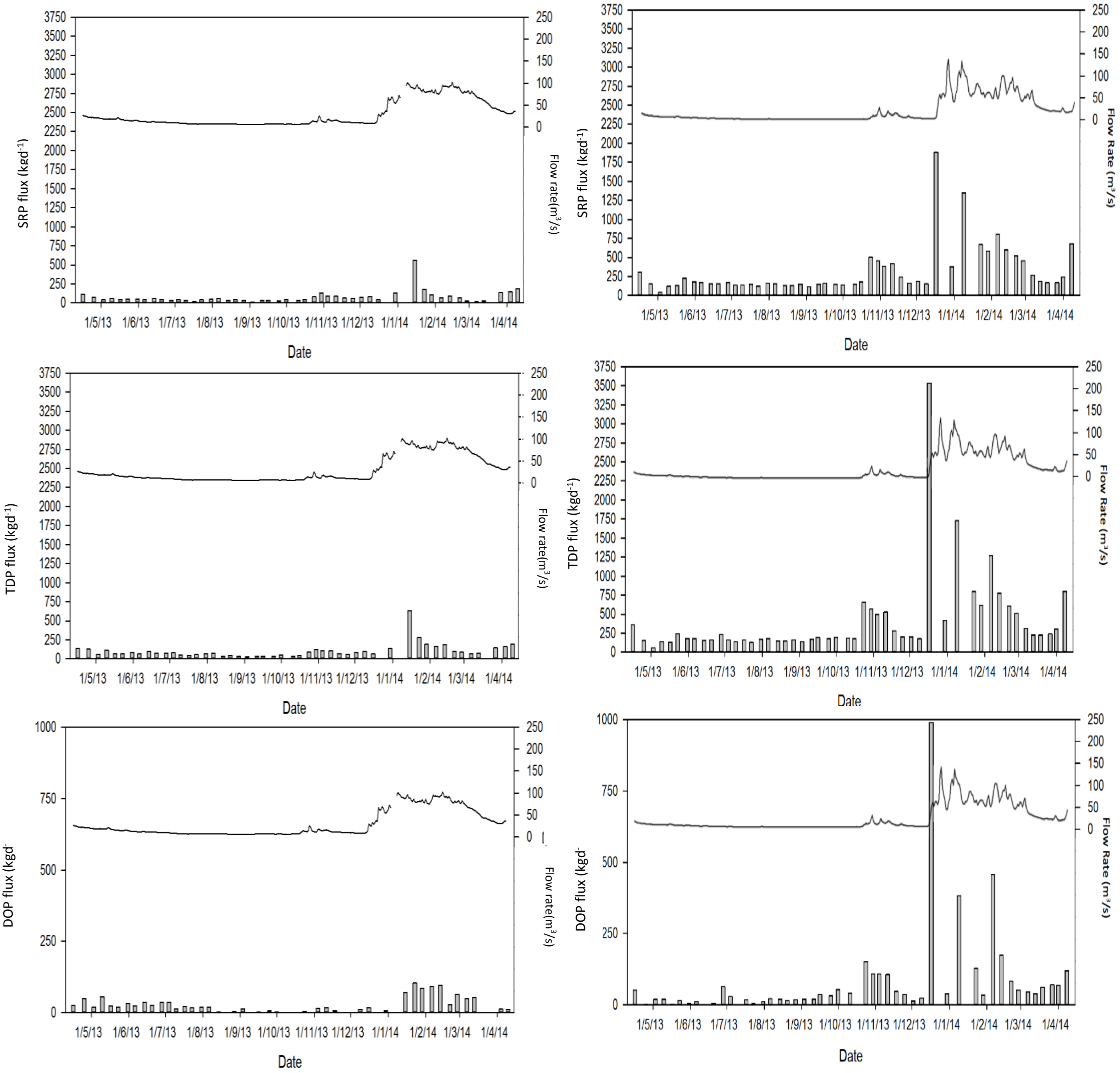


Figure 4.6: Fluxes of SRP, TDP and DOP from Knapp Mill on River Avon (left panels) and Throop on River Stour (right panels) over the sampling period. River flow is shown from the gauging stations at Throop and Knapp Mill

4.3.5 Relationships between phosphorus and environmental parameters

Relationships between TDP, SRP and DOP and environmental data collected at the four sites were investigated using correlation analyses (Table 4.2). At all of the sites there was a significant positive correlation between both TDP and DOP with temperature. There was also a positive correlation between SRP and temperature at all sites except at Knapp Mill. There is also a positive correlation between TDP, SRP and DOP and conductivity at all of the sites except Knapp Mill where no correlations were evident. There was a positive correlation between TDP, SRP and DOP with salinity at Mudeford. There was a positive correlation between SRP and DOP with pH, a significant negative correlation between SRP and chlorophyll a and a positive correlation between DOP and chlorophyll a concentration at Knapp Mill. There is a significant correlation between oxygen saturation and TDP, SRP and DOP at Mudeford. There were no other significant correlations observed. River flow data is only available for Knapp Mill and Throop, so the flow rate at the site Iford Bridge was assumed to be the same as Throop and the flow rate at Mudeford Quay was assumed to be the sum of the river flow rates at Knapp Mill and Throop. Significant negative correlations were observed With TDP and SRP and river flow at both sites. Detailed information on the relationships between the phosphorus fractions and the environmental parameters can be found in Appendix 1.

Table 4.2: Relationships between, TDP, SRP and DOP and physiochemical parameters (Appendix 1). All parameters were measured using a YSI EXO 2 sonde with the exception of flow rate which measured by the Environment agency. Any values highlighted in red are significant (p-value<0.05)

		Knapp Mill			Throop			Iford			Mudeford		
		TDP	SRP	DOP	TDP	SRP	DOP	TDP	SRP	DOP	TDP	SRP	DOP
Temperature (°C)	Pearsons correlation	0.35	0.13	0.50	0.59	0.68	0.09	0.73	0.77	0.37	0.75	0.44	0.50
	p-value	0.01	0.36	0.00	0.00	0.00	0.52	0.00	0.00	0.02	0.00	0.00	0.00
	N	48	48	48	49	49	49	39	39	39	48	48	48
Conductivity (mS/cm)	Pearsons correlation	0.20	0.07	0.27	0.55	0.66	0.02	0.69	0.74	0.33	0.61	0.43	0.60
	p-value	0.16	0.65	0.06	0.00	0.00	0.90	0.00	0.00	0.04	0.00	0.00	0.00
	N	49	49	48	49	49	49	39	39	39	49	49	49
Salinity	Pearsons correlation	-	-	-	-	-	-	-	-	-	0.554	0.404	0.536
	p-value	-	-	-	-	-	-	-	-	-	0.00	0.00	0.00
	N	-	-	-	-	-	-	-	-	-	48	48	48
pH	Pearsons correlation	-0.188	0.353	0.322	-0.094	-0.019	-0.273	-0.073	0.014	-0.186	0.111	-0.183	0.282
	p-value	0.2	0.014	0.025	0.519	0.896	0.058	0.658	0.934	0.257	0.454	0.213	0.052
	N	48	48	48	49	49	49	39	39	39	48	48	48
Chlorophyll a (µg/L)	Pearsons correlation	-0.188	-0.407	0.481	-0.253	-0.263	-0.126	0.055	0.083	-0.012	0.561	0.282	0.626
	p-value	0.2	0.005	0.001	0.08	0.068	0.388	0.739	0.616	0.94	0.12	0.058	0.01
	N	48	46	46	0.49	49	49	39	39	39	46	46	46
Oxygen saturation (%)	Pearsons correlation	0.231	0.109	0.236	0.086	0.11	-0.016	-0.064	-0.039	-0.079	0.489	0.346	0.48
	p-value	0.114	0.459	0.106	0.557	0.451	0.913	0.709	0.822	0.648	0.000	0.016	0.001
	N	48	48	48	49	49	49	36	36	36	48	48	48
Flow rate (m^3/s)	Pearsons correlation	-0.694	-0.621	-0.196	-0.560	-0.652	-0.087	-0.662	-0.794	-0.193	-0.603	-0.563	-0.498
	p-value	0.000	0.000	0.181	0.000	0.000	0.558	0.000	0.000	0.240	0.000	0.000	0.000
	N	49	49	48	48	48	48	39	39	39	50	50	50

4.3.6 Estuarine transects

Mudeford Quay, site 1, the closest site to the mouth of the estuary had the lowest mean TDP and SRP concentration of all of the sample sites (Table 4.3). The highest mean TDP and SRP concentration from the 6 sites was at Christchurch Quay, site 5. The TDP and SRP concentration was higher at sites 5 and 6, further from the mouth of the estuary, than at the other 4 sites on every date sampled (Table 4.3). The DOP concentration was also highest at site 5 on the last 3 sampling dates; however, unlike the TDP and SRP concentration, The DOP concentration on the 12 and the 25 July 2013 was more homogeneous between sites, with a lower DOP concentration at sites 4 and 5.

Table 4.3: Concentrations of SRP, TDP and DOP at the riverine transect sites

		1 Run at Mudeford	2 Ferry Pontoon	3 Blackberry Point	4 Grimbury Marsh	5 Christchurch Quay	6 Tuckton Bridge
SRP	Max (µM)	2.31	3.94	2.34	3.97	11.35	16.09
	Min (µM)	0.31	0.77	0.84	0.83	7.97	6.43
	Mean (µM)	1.15	2.17	1.41	2.23	9.92	11.01
	Standard deviation	0.75	1.47	0.56	1.15	1.49	3.79
TDP	Max (µM)	3.03	4.35	2.96	5.68	22.49	20.25
	Min (µM)	1.26	2.19	0.98	0.85	9.63	6.90
	Mean (µM)	2.14	3.10	2.27	2.91	13.80	12.17
	Standard deviation	0.72	0.88	0.86	1.86	5.03	4.99
DOP	Max (µM)	1.96	1.94	1.66	1.71	1.77	4.16
	Min (µM)	-0.08	-0.07	0.15	0.02	0.82	-0.44
	Mean (µM)	1.00	0.93	0.86	0.68	1.21	1.15
	Standard deviation	0.94	0.80	0.70	0.76	0.41	1.81

On the 12/6/14 the TDP concentration ranged from 2.89µM at site 3 to 9.63µM at site 5, the SRP concentration from 1.23µM at site 3 and 8.81µM at site 5 and the DOP concentration ranged from 0.82µM at site 5 and 1.96µM at site 2 (Figure 4.7). On 25/6/14, the TDP concentration ranged from 2.3µM at site 1 and 12.5µM at site 5, the SRP concentration ranged from 12.8µM at site 6 and 0.3µM at site 1 and the DOP concentration ranged from undetectable concentrations at site 6 and 2.0µM at site 11. On 10/7/14 the TDP concentration ranged from 13.0µM at site 5 and 0.9µM at site 4, the SRP concentration ranged from 11.5µM at site 6 and 0.8µM at site 4 and the DOP concentration ranged from 0.12µM at site 3 and 1.8µM at site 5. On 24/7/14 the TDP concentration ranged from 22.5µM at site 5 and 1.6µM at site 1, the SRP concentration ranged from 0.7µM at site 1 and 16.1µM at site 6 and the DOP concentrations ranged from 0.1µM at site 4 and 4.2µM at site 6. The TDP concentration on 21/8/14 ranged from 11.3µM at site 5 and 1.3µM at site 1, the SRP concentration ranged from 10.3µM at site 5 and 1.3µM at site 1 and the DOP concentration ranged from undetectable concentrations at sites 1 and 2, and 1.1µM at site 5 (Figure 4.7).

The TDP concentration was higher at sites 5 and 6 than at the other 4 sites on every date sampled (Table 4.3, Figure 4.7). Highest TDP concentration was seen at site 1, the closest site to the mouth of the estuary was the lowest on average of all of the sample sites. The highest mean TDP concentration of the 6 sites is at Christchurch Quay. The highest TDP concentration was observed at site 1, the closest site to the mouth of the estuary was the lowest on average of all of the sample sites. The highest mean TDP concentration of the 6 sites is at Christchurch Quay site 5. The TDP concentration was higher at sites 5 and 6 than at the other 4 sites on every date sampled (Table 4.3).

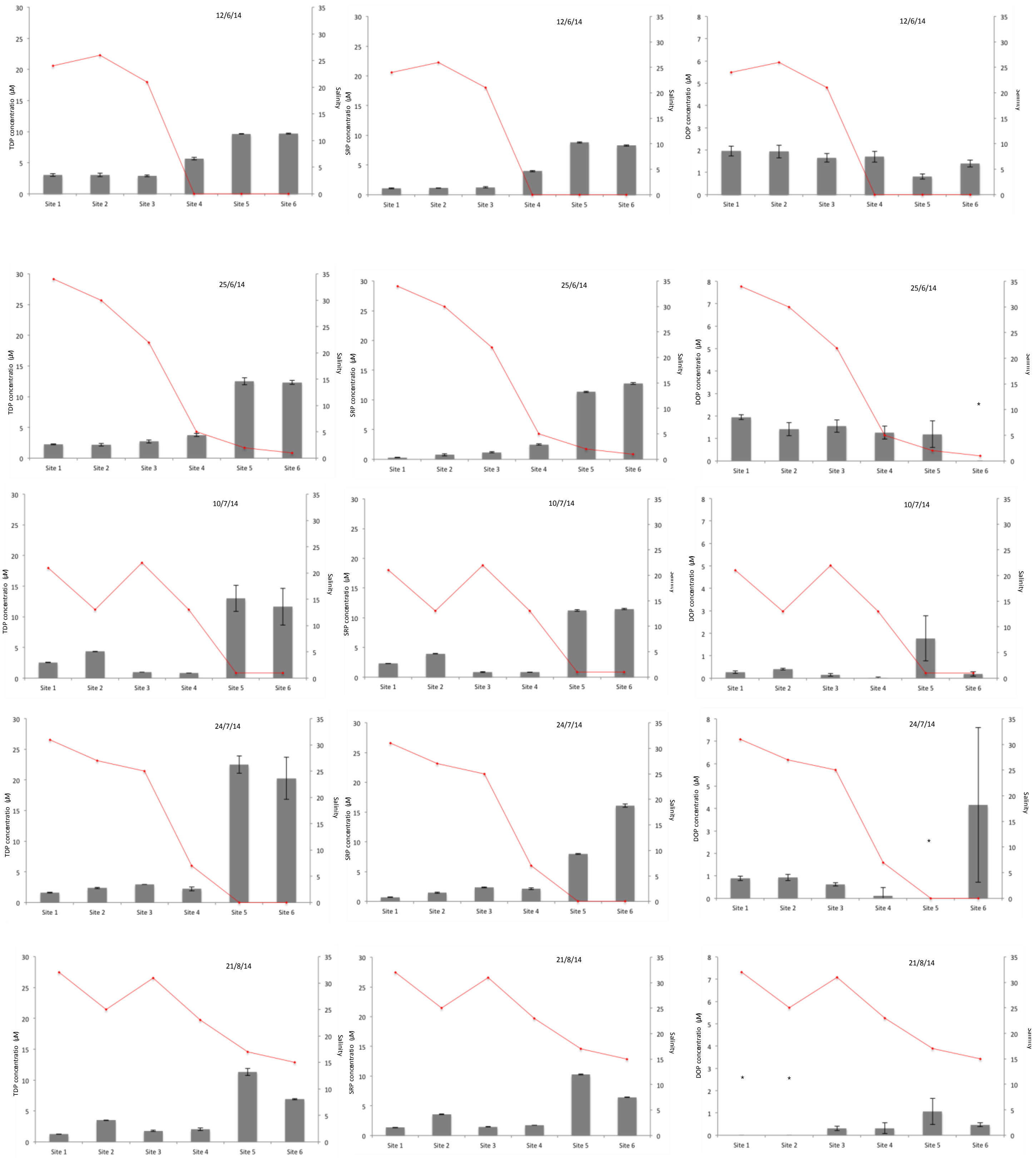


Figure 4.7: Concentrations of TDP (left column), SRP (middle column) and DOP (right column) at the sampling points from the transect analysis (Figure 2) on the 5 dates in which transect analyses were done. Grey bars show P concentration in µM and red lines show salinity. Error bars show 1 sd after 3 replicates.

Christchurch Harbour is an estuarine system with seawater mixing with freshwater inputs from the River Avon and River Stour. This means that the relationships between nutrients and changing salinity in the estuary are important factors to consider. Figures 4.8 and 4.9 show the relationships between salinity and the P fractions measured at the 5 dates in which transects of the estuary were undertaken. SRP and TDP concentrations decrease with salinity (Figure 4.7), a trend seen throughout the year with the routine spot samples. The TDP and SRP also both show non- conservative mixing behaviour in the estuary with respect to salinity (Figure 4.8). The DOP concentrations appeared to be less affected by salinity and remained relatively constant. However, at extremely low salinities the DOP concentration is higher than at higher salinities. The proportion of TDP that is DOP increased with increasing salinity (Figure 4.9, Appendix 1) on every date sampled during this investigation.

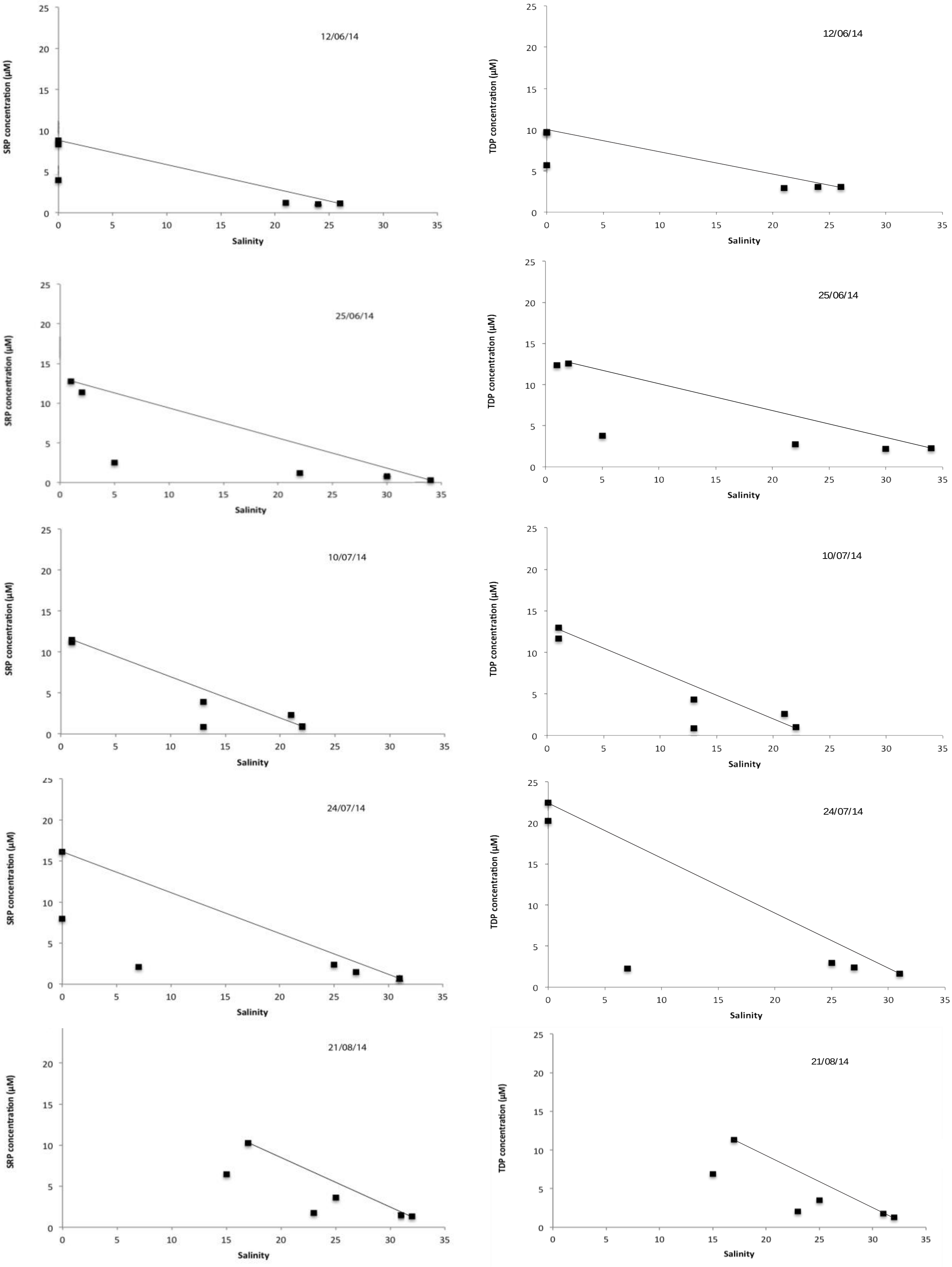


Figure 4.8: Relationships between SRP (left plots), TDP (right plots) and salinity at sites 1-6 on the 5 dates in which transect samples were analysed. Lines indicate mixing between maximum concentrations at zero salinity and minimum P concentrations at maximum salinity values

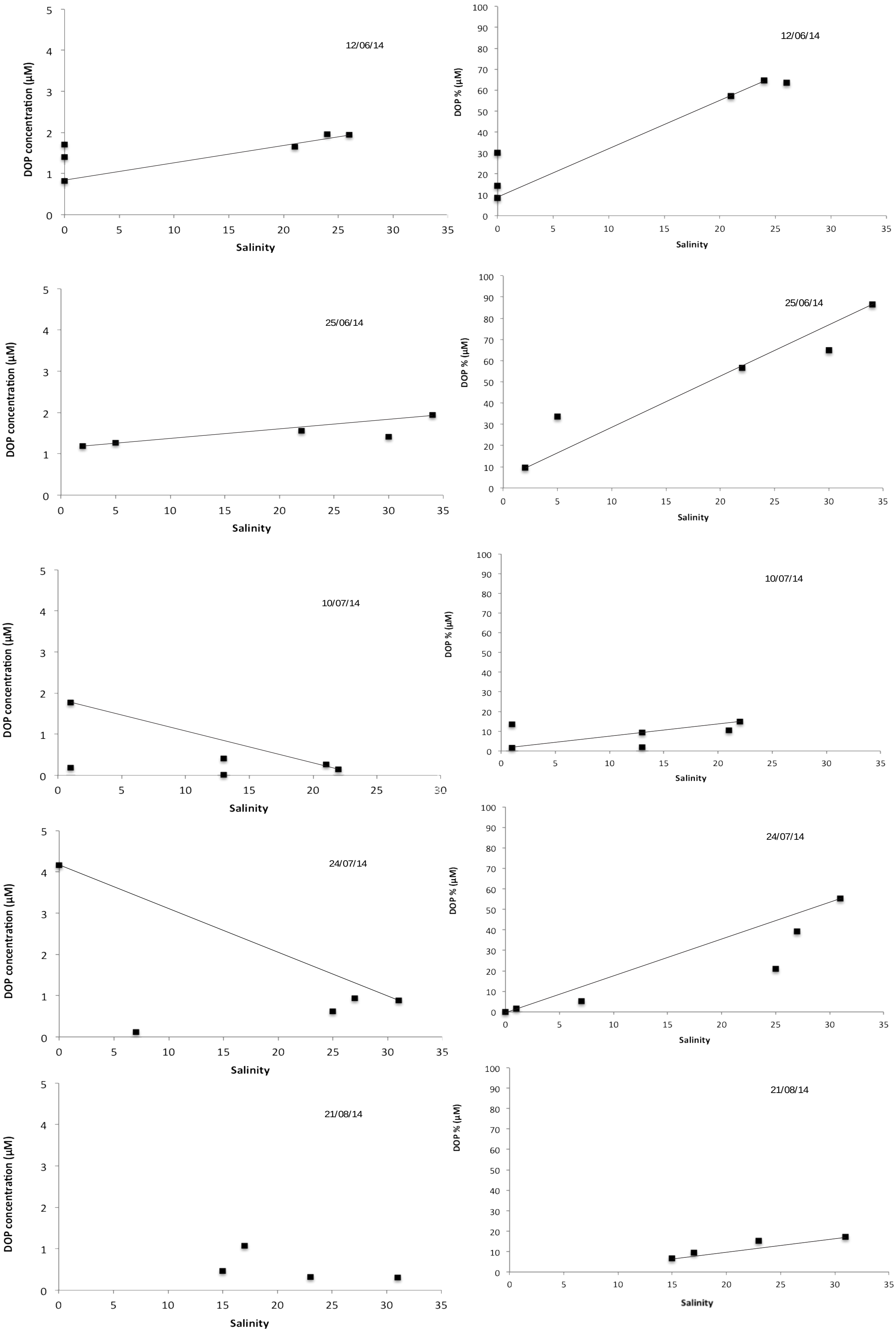


Figure 4.9: Relationships between DOP (left plots), DOP % (Right plots) and salinity at sites 1-6 on the 5 dates in which transect samples were analysed. Lines indicate mixing between maximum concentrations at zero salinity and minimum P concentrations at maximum salinity values.

4.4 Discussion

4.4.1 Seasonal nutrient dynamics

The annual monitoring of three riverine sites (Knapp Mill, Throop and Iford) and the associated estuary (Christchurch Harbour) on a weekly basis has produced a new understanding of the fluxes and concentrations of DOP, SRP and TDP in the river and estuary system.

SRP was shown to be a significant fraction of the dissolved phosphorus in the rivers analysed in this study. However, there was significant variation in SRP concentration and flux at different sites and at different times of the year. The yearly average SRP concentration was lower at Knapp Mill on the River Avon (1.9µM) than at Throop (10.9µM) or Iford (15µM) on the River Stour or at Mudeford in Christchurch Harbour (5.7µM). Short-term fluctuations of nutrients are a common feature of lowland river and estuary systems (Zwolsman, 1994) but the seasonal variability of phosphorus in the rivers studied in this investigation is too large to be explained by random fluctuations in river chemistry.

Nutrient concentrations in river and estuary systems are influenced by a number of factors, including the geology of the catchment. In pristine rivers, annual loads of phosphorus are extremely low, since phosphorus is not available from the majority of natural geologies, particularly in the UK (Mainstone and Parr, 2002). However, this is altered in lowland rivers, where human activities have increased the amount of phosphorus entering river systems.

One possible reason for the elevated phosphorus concentrations on the River Stour compared to the River Avon is related to differences in catchment geology of the two catchments. The upper Stour catchment is associated with several clay-influenced tributaries (including the Bow, Filley and Caudle Brooks, the Cam, the Lydden and the Divelish) whereas the River Avon is a groundwater-dominated system with a chalk-based upper catchment. Previous studies have indicated that low natural concentrations of phosphorus in calcareous aquifers give rise to low (<0.4µM) riverine concentrations (Mullholland and Hill, 1997; Mainstone et al., 1999). As the chalk aquifers in the River Avon catchment have a large storage capacity (Whitehead et al., 2014), this will result in low phosphorus concentrations, which have been observed in previous studies (Jarvie et al., 2005). In contrast, clay based catchments may produce higher natural concentrations of total phosphorus than sand and chalk-based catchments, such as the river Avon, due to the dominance of run-off as a hydrological pathway (Mainstone and Parr, 2002). In addition to this, coarse textured soils such as sand generally hold less phosphate due to the more inert character of sand particles compared to clay. Therefore, the predominantly clay based catchment of the River Stour may contribute to the higher concentrations of SRP detected at Throop and Iford compared to Knapp Mill on the River Avon.

DOP is an important component of the total phosphorus pool because it is bioavailable to phytoplankton (Smith et al., 1986; Mather et al., 2008; Lomas et al., 2010), present in sewage effluent (Servais et al., 1999) and is therefore a potential major contributor to eutrophication in coastal waters. Numerous studies have demonstrated an accumulation of dissolved organic matter after phytoplankton blooms in a variety of marine (Williams, 1995; Wetz and Wheeler, 2003; Suksomjit et al., 2009) and riverine (Baines and Pace, 1991) systems. At Knapp Mill on the River Avon there was a peak in DOP concentration from May 2013 to August 2013 and a positive correlation between DOP and chlorophyll a concentration is evident (Table 4.2, Appendix 1.1.3). This suggests that DOP concentrations in the River Avon at this time were associated with a phytoplankton bloom. At Throop, this peak in DOP in not seen but a peak in DOP occurs in October indicating that different communities of phytoplankton may be present in the two rivers. Mudeford Quay in Christchurch Harbour also had a distinctive peak in DOP from May 2013 to October 2013. Phytoplankton communities can develop in estuaries with long residence times (Crump et al., 2004) or when water temperatures are high (Muylaert et al., 2000) plus estuarine plankton can be influenced by both marine and riverine phytoplankton communities. In this investigation, the concentration of DOP was higher at Mudeford than the sum of the DOP detected at Knapp Mill and Iford and also, DOP and chlorophyll a concentration were positively correlated in the estuary (Table 4.2 Appendix 1.4.3). This indicates that there is either a distinct phytoplankton community in the estuary or that the phytoplankton community in the estuary is influenced by plankton from the English Channel.

The DOP and SRP concentration at Iford Bridge was elevated throughout the year compared to Throop and there are a number of spikes in DOP and SRP concentration at Iford Bridge which were not present in samples collected on the same day at Throop, despite them both being sites on the River Stour. One reason for this may be the presence of Holdenhurst sewage treatment works (STW), an STW that serves approximately 160,000 people in the Bournemouth and Christchurch area. The average SRP and DOP concentrations at Iford Bridge were 30.9µm and 21.0µM compared to 22.3µm and 12.1µm at Throop respectively. The DOP and SRP spikes associated with the sewage outflow at Holdenhurst are also evident at the site at Mudeford Quay. Indicating that the sewage outflows in lowland rivers can significantly alter the P dynamics in estuarine and coastal waters. Jarvie et al., (2005) monitored 45 lowland river catchments across the UK in relation to the relative importance of point and diffuse sources using boron as a tracer of sewage. Point source inputs to the studied catchments were identified to pose a more significant risk to eutrophication than diffuse sources, even in agricultural areas. Other investigations have observed similar effects of point source contamination of rivers with regard to elevated concentrations of various phosphorus fractions (Demars and Harper, 1998; Maya et al., 2007; Calijuri et al., 2008).

This investigation showed that a range of TDP, SRP and DOP concentrations are present at the river and estuary sites, dependent on a number of factors such as season, flow rate and the influence of point source inputs. The SRP and DOP concentrations at Knapp Mill were low, comparable to concentrations in a number of rivers in South Korea and India (Table 4.4). Compared to other rivers in the UK, the max concentration of DOP and SRP at Knapp Mill is low, indicating relatively good water quality in the river Avon. The DOP concentration in the River Stour at Iford was higher than all of the studies listed in Table 1 with the exception of the River Kennet at Stitcombe Mill, which is another site a few kilometres downstream from a sewage treatment works (Neal et al., 2000b). This indicates that the elevated phosphorus concentrations at Iford are partly due to sewage treatment works effluent and that the issue of sewage effluent is an environmental issue affecting other riverine systems in the UK. The DOP concentration in the River Stour at Throop was lower than in the River Kennet and at Iford Bridge, with similar to concentrations on the River Wear at Sunderland Bridge, a river with a number of point source inputs into the system, (Neal et al., 2000). The phosphorus concentrations at Mudeford Quay were higher than the other estuarine studies in Table 4.5. This may be due to a number of reasons including, differences in nutrient load to the estuary, residence time of the estuary and the salinity of the samples at the time of sampling. This comparison highlights a number of issues with estuarine research and the ability to draw comparisons between studies. Estuarine systems are dynamic, each with unique features influenced by both tides and river flow. Therefore, any comparison of nutrient concentrations need to be standardised as far as possible. Future research needs to focus on using standardised techniques to analyse phosphorus fractions in environmental water samples if reliable data are to be compared and used for future planning.

Table 4.4: TDP, SRP and DOP concentrations at a number of riverine sites from this current investigation and other studies.

					TDP (µM)			SRP (µM)			DOP (µM)			DOP %			
River	**Location**	**Length (km)**	**Catchment Area (km^2)**	**Mean Annual Flow Rate (m^3/sec)**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Reference**
River Avon, Knapp Mill	Hampshire, UK	96	1750	27	2.1	0.4	3.8	1.6	0.1	3.3	0.5	BDL	1.5	27.5	BDL	75.1	Current Study *
River Stour, Throop	Hampshire, UK	97	1240	20	12.9	2.6	28.4	10.9	1.7	22.2	2	BDL	12.1	15.5	0.2	46.9	Current Study *
River Stour, Iford	Hampshire, UK	97	1240	-	17.7	3.4	43.8	14.9	1.3	30.8	2.8	BDL	21	14.5	BDL	65	Current Study *
Wear, Sunderland Bridge	Northumberland, UK	110	658	10	12.5	-	-	10.5	-	-	2.1	-	-	16.5	-	-	Neal et al., 2000a **
Tweed, Norham	Northumberland, UK	156	5000	78	3.9	-	-	1	-	-	2.9	-	-	75	-	-	Neal et al., 2000a **
Nidd	North Yorkshire, UK	94	-	-	7.1	-	-	4.8	-	-	2.3	-	-	31.8	-	-	Neal et al., 2000a **
Ouse	North Yorkshire, UK	84	3000	12	4.8	-	-	2.6	-	-	2.3	-	-	46.7	-	-	Neal et al., 2000a **
Thames, Wallingford	Oxfordshire, UK	346	16133	66	4	-	-	3.4	-	-	0.6	-	-	14.5	-	-	Neal et al., 2000a **
Kennet, Stitcombe Mill	Oxfordshire, UK	72	1164	10	16.3	13.2	19.9	14.7	11.5	19.4	1.6	-	-	10.1	-	-	Neal et al, 2000b **
Tamar River	Devon, UK	34	924	34	1.6	1.4	1.9	1.3	1.1	1.4	0.4	0.1	0.7	23.5	8	36	Monbet et al., 2009 †
Han	Gangwon, S. Korea	514	26018	670	5.8	-	-	5.6	-	-	0.2	-	-	3	-	-	Islam et al., 2013 **
Kum	N. Cholla, S. Korea	401	9859	-	4.6	-	-	4.3	-	-	0.3	-	-	7.6	-	-	Islam et al., 2013 **
Youngsan	N. Cholla, S. Korea	115	3371	-	5.2	-	-	4.8	-	-	0.4	-	-	6.9	-	-	Islam et al., 2013 **
Periyar River	Kerala State, India	244	5398	295	2.6	-	-	1.3	-	-	1.2	-	-	48.1	-	-	Maya et al., 2007 **
Chalakudy River	Kerala State, India	130	1250	52	2.3	-	-	1.3	-	-	1	-	-	44.4	-	-	Maya et al., 2007 **
Jiulong River	Fujian Province, China	258	14,700	-	2.6	-	-	2.1	-	-	0.5	-	-	18	-	-	Lin et al., 2013 **

* TDP determined using ICP-MS.

*** TDP determined using high temperature combustion.

** TDP determined using chemical oxidation.

⍰ TDP determined using Enzymatic hydrolysis.

Table 4.5: TDP, SRP and DOP concentrations at a number of riverine sites from this current investigation and other studies.

					TDP (µM)			SRP (µM)			DOP (µM)			DOP %			
Estuary	**Location**	**Length (km)**	**Catchment Area (km^2)**	**Mean Annual Flow Rate (m^3/sec)**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Reference**
Christchurch Harbour, Mudeford	Dorset, UK	-	2990	-	10.4	1.1	28.2	5.7	1.1	17	4.7	BDL	17.8	37.9	2.8	68.1	Current Study *
Tamar estuary	Devon, UK	34	924	34	1.8	1.6	2	1.3	31	50	0.5	0.4	0.7	29.8	20	40	Monbet et al., 2009 †
Periyar Estuary	Kerala State, India	244	5398	295	3.7	-	-	1.9	-	-	1.8	-	-	49.6	-	-	Maya et al., 2007 **
Chalakudy Estuary	Kerala State, India	130	1250	52	3.7	-	-	2.6	-	-	1.2	-	-	31.3	-	-	Maya et al., 2007 **
Tamshui estuary	Tamsui, N. Taiwan	159	2726	226	0.2	-	-	0.2	-	-	0.1	-	-	24.3	-	-	Fang. 2000 ***
Jiulong estuary	Fujian Province, China	258	14700	-	1.3	-	-	0.7	-	-	0.6	-	-	47.3	-	-	Lin et al., 2013 **

* TDP determined using ICP-MS.

** TDP determined using chemical oxidation.

*** TDP determined using high temperature combustion.

† TDP determined using Enzymatic hydrolysis.

4.4.2 Estuary processes

The sum of the TDP and the SRP concentrations measured at Throop and Knapp Mill was greater than the TDP and SRP concentrations detected in water collected from Mudeford Quay. In addition to the riverine inputs at Christchurch Harbour from the Stour and Avon, there are other potential inputs from small stream inputs such as the river Mud, suggesting that there is removal of these P fractions within the estuary. However, as the TDP concentration was lower in the estuary than the combined concentration from the river sites, phosphorus is likely being removed into the sediments or into the particulate fraction of P in the estuary. As water enters at higher salinities, flocs can form in the water column, binding phosphorus into particles (Forsgren et al., 1996). These particles can then get resuspended with increasing shear forces due to factors such as increased flow (Corbett 2010). However, as all water samples were filtered prior to analysis during this investigation and particulate phosphorus was not included in the measured fractions.

Water samples collected at Mudeford Quay are affected by riverine inputs as well as high salinity inputs from adjacent coastal seawater. Over the period 16/4/13 to 30/12/13 the salinity at Mudeford Quay ranged from 33.9 and 1.2 whereas between 7/1/14 to 13/3/14 the salinity at Mudeford Quay was consistently less than 0.4, lower than at any point during the whole period of sampling. Over this wet winter period, the average monthly rainfall was 161.4mm compared with 66.5mm over the rest of the sampling period, leading to Christchurch Harbour becoming almost a freshwater lake. The estuarine transects that were undertaken over summer 2014 show a distinct salt intrusion into the estuary. However, this salt intrusion is not evident when the flow rate of the rivers is high from December 2013 to March 2014, evidenced by the sustained low salinities at Mudeford Quay. Due to the removal of phosphorus in waters with a high salinity (Forsgren et al., 1996), the lack of a saline input over these winter months mean that the estuarine sites are exposed to higher phosphorus concentrations than during reduced flow conditions.

Transects of Christchurch Harbour estuary were sampled at over the summer in 2014. Water samples collected during summer transects of Christchurch Harbour contained higher concentrations of DOP and SRP at sites further up the estuary, with decreasing concentrations towards the mouth of the estuary at Mudeford Quay. However, at site 2, there is often a slight increase in TP and SRP concentration. Despite this, overall the variation of SRP across the salinity gradient showed strong removal behaviour as has been observed in a number of estuarine studies (Pitkanen et al., 1995; Uncles et al., 2002).

At low salinities (<6), the ionic strength in rivers increases with increases in salinity (Meng et al., 2014). Dissolved P may then be encapsulated in newly formed aggregates and removed from the water column as these aggregates sink (Forsgren et al., 1996). As the salinity increases further, a larger amount of anions (Cl^-,SO_4^{2-}, HCO^{3-}, OH^- and Br^-) compete for adsorption sites on the flocs , decreasing adsorption of SRP and causing a release of sorbed SRP into the water (Wang et al., 2006; Millero et al., 2001; Zhang and Huang, 2011). This may explain the slight increase in TP and SRP in the higher salinity regions close to Mudeford Quay.

However, SRP and DOP concentrations showed non-conservative removal behaviour with respect to salinity suggesting that there are other removal processes in the estuary in addition to the saline mixing. The role of suspended particles and estuarine sediments on the fate of the phosphorus fractions analysed have not been examined as part of this study, but it is clear that there is some removal of P between the lowest gauging stations on the two rivers and the estuary mouth. Phosphorus dynamics in estuarine systems are complex (Statham, 2012) and release of P from particles due to ionic interactions with the particles has been observed which may explain the SRP increase at site 2 in this investigation (Table 4.3). However, the non-conservative mixing behaviour of SRP and TDP with saline water indicates that P is settling to the floor of the estuary to form a reservoir of P within the sediments (Wang and Li, 2010). However in addition to this, there may be biological removal of P via phytoplankton uptake and bacterial activity.

4.4.3 High flow events

At present day levels, about 18% of the moderate daily precipitation extremes over land are attributable to anthropogenic global temperature increases since pre-industrial times (Fischer et al,. 2015). As a result of this, the likelihood of stochastic storm events are predicted to increase in the future due to climate change (IPCC, 2007).

The sampling from April 2013 to April 2014 was typified by low river flow conditions in summer followed by a large, sustained period of rainfall and high flow conditions over winter. At each river site there is a large spike in TDP, SRP and DOP concentrations on the 16/12/13, corresponding to the start of the large rainfall event.

Storm events generate significant movement of nutrients and sediments in river catchments (Arreghini et al., 2005). A large proportion of a catchments annual phosphorus transport can be generated by a small number of large storm events (Jarvie et al., 2002; Jordan et a., 2007; Lloyd et al 2016). Traditionally, parameters such as turbidity have been used to indicate storm events in rivers. This is because they can be measured in-situ at high frequency and have been shown to be suitable proxies for the transport of sediment and sediment bound nutrients such as nitrates and phosphates (Stubblefield et al., 2007). By examining the relationship between river discharge and water quality parameters such as turbidity, nitrate, phosphate, pH and conductivity it is possible to observe cyclical hysteresis. These hysteresis loops were classified by Williams, (1989) into 5 classes. Class 1 was described as a single valued line, where the increases and decreases in river flow and sediment concentrations are synchronized, caused by high sediment concentrations. Class 2 is a clockwise loop where sediment concentrations peak early in the high river flow event, caused by flushing of sediment, which may become exhausted by the end of the event. Class 3 is an anticlockwise loop, where the sediment concentration peak lags behind the increase in flow, potentially signifying differences in transit times between water and sediment. Class 4 is a combination of classes 1 and 2, described as resulting from a change in the relationship during a storm event, potentially due to changes in the availability, form or storage of sediment. The final class (5) was described as a figure of eight configuration, combining classes 2 and 3, again due to changes in the availability, form or storage of sediment. These hysteresis loops described by Williams, (1989) are not a perfect system for classifying hysteresis loops as many systems may not fit into these classifications and the loops may occur for different reasons outlined in this paper. However, the study of discharge-water quality hysteresis in storm events can provide a valuable research tool to infer the likely contributing source areas and flow pathways contributing to nutrient and sediment transport in catchments (Lloyd et al., 2016).

The sampling period of this investigation extended over the wettest January since 1910 (Slingo et al., 2014), with extended flooding over the South of England. This presented a unique opportunity to examine phosphorus dynamics over a time of intense rainfall and river flow. There were two high flow events, one in October, which lasted for 4 weeks and a much larger event from January 2014 to March 2014. During these periods it is possible to identify hysteresis loops identified by Williams, (1989). During the October event, turbidity exhibits class 1 behaviour, increasing and decreasing with river flow (Chapter 2, figure 2.2-2.5). However, during the extended flow conditions of early 2014, there is a large peak in turbidity followed by a decrease in turbidity that is not synchronous with flow rate. This indicates that the particulate load in the River Avon and the River Stour is limited and is depleted with extended high flow events. In this case, by using turbidity as a proxy for sediment concentration it is possible to infer a number of conclusions about the phosphorus speciation in the Christchurch Harbour river catchments with regard to storm events. At Throop on the River

Stour, there is a large peak in TDP on 16/12/13; this then rapidly decreases with increased river flow, exhibiting class 1 hysteresis behaviour. However, this large peak is not seen in SRP, indicating that this large peak in TDP is composed of particle/colloidal-associated P.

At Knapp Mill on the River Avon, TDP concentration responds differently than at Throop on the river Stour. The increase in TDP concentration is less pronounced and is before the increase in flow rate, decreasing at a slower rate than at Throop. This also indicates class 1 hysteresis behaviour, but the difference in peak shape infers differences in the sediment structure between the two catchments. TDP and SRP at all of the river sites show features of class 1 hysteresis when exposed to the high flow event in October 2013, in similarity to the turbidity profile. This indicates that the river does not become depleted in nutrients until there is a significant increase in river flow, as seen in early 2014.

These results indicate that a long period of low rainfall on soils with low permeability, such as the clay soils in the River Stour catchment, followed by intense rainfall, could favour movement of dissolved phosphorus in river systems. During this period, leaf litter decomposition might have initially released nutrients, which were then transported to the stream by leaching (Arreghini et al., 2005).

In addition to the absolute P concentrations described in the previous section, fluxes of the 3 analysed fractions of P from the two rivers were calculated using daily mean river flow data provided by the Environment Agency. SRP and TDP fluxes were consistently higher in the River Stour than the river Avon despite the typically low flow conditions. This indicates that the river Stour contributes more P in all of the analysed fractions to the Christchurch Harbour estuary than the River Stour, even during high flow events.

During high flow conditions, the fluxes of both SRP and TDP increased. This has been observed in previous studies, in addition to similar patterns for nitrate and dissolved silicon Sigleo and Frick, 2003, Arreghini et al., 2005). Schönbrunner et al., (2012) found that periods of drying followed by flood events lead to increased delivery of phosphorus into riverine systems from flood plains. In this investigation approximately 50% of the annual flux of TDP, SRP and DOP passed through Knapp Mill and Throop during the high flow event in early 2014, with the exception of DOP at Throop. This indicates that cyclical dry and wet periods result in a large release of P during the first high rainfall event post-dry period.

4.4.4 EU Water Framework directive

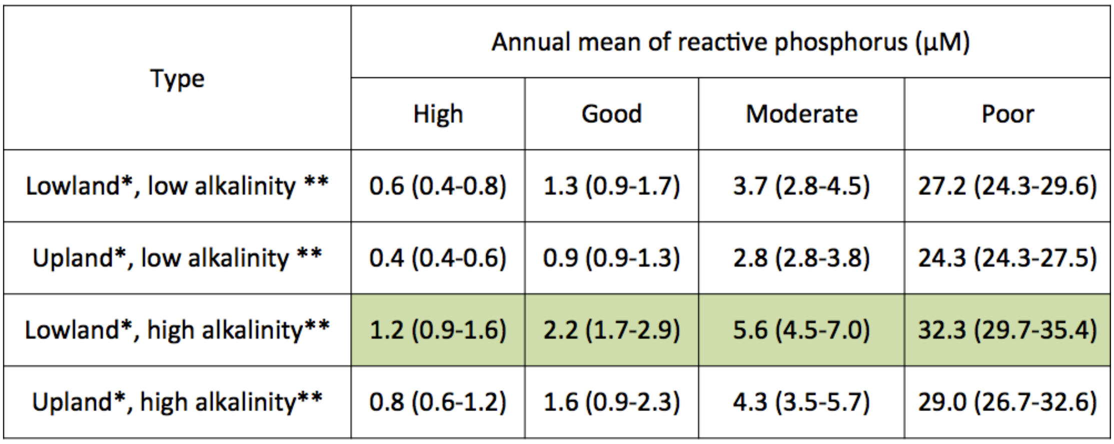
The European Union's Water Framework Directive (WFD: CEC, 2000) requires, where feasible, good water quality in terms of biology in order to address eutrophication for “high risk” lowland areas (Mainstone et al., 2000; Mainstone and Parr, 2002; Neal et al 2010). (Table 4.7). The WFD has a guide to phosphorus standards in lowland river systems, such as the River Stour and the River Avon. The classifications for the standards for SRP are shown in (Table 4.4).

Table 4.6: Proportion of samples, which were categorised as poor, moderate, good of high with regard to the EU Water Framework Directive, CEC (2000)

	Knapp Mill	Throop	Iford	Mudeford
% High	75	2	2.5	18
% Good	26	32	20	32
%Moderate	0	66	77.5	50
% Poor	0	0	0	0

Knapp Mill was classified as good or moderate with regard to SRP for 100% of the sampling period (Table 4.6). At Throop and Iford the water quality with regard to SRP was the worst with 66% of the samples collected at Throop classified as moderate and 77.5% at Iford. Only 32% of the time Throop was classified as good and it was only classified as high 2% of the time. Iford was classified as good only 20% of the time and as high for 2.5% of the time. Mudeford was classified as moderate for 50%, good for 32% and high for only 18%. However, at no point did the phosphorus concentration at any site reach the ‘poor’ standard according to the WFD.

Table 4.7: Boundaries for water quality classifications as described by the EU Water Framework Directive (2000). The River Stour and Avon both fall into the ‘Lowland’, ‘high alkalinity’ category.



Type	Annual mean of reactive phosphorus (µM)			
	High	Good	Moderate	Poor
Lowland*, low alkalinity **	0.6 (0.4-0.8)	1.3 (0.9-1.7)	3.7 (2.8-4.5)	27.2 (24.3-29.6)
Upland*, low alkalinity **	0.4 (0.4-0.6)	0.9 (0.9-1.3)	2.8 (2.8-3.8)	24.3 (24.3-27.5)
Lowland*, high alkalinity**	1.2 (0.9-1.6)	2.2 (1.7-2.9)	5.6 (4.5-7.0)	32.3 (29.7-35.4)
Upland*, high alkalinity**	0.8 (0.6-1.2)	1.6 (0.9-2.3)	4.3 (3.5-5.7)	29.0 (26.7-32.6)

*"Lowland" means less than or equal to 80 metres above mean sea level and "Upland" means more than 80 metres above mean sea level.

** "Low alkalinity" with a concentration CaCO3 of less than 50 mg per litre and "High alkalinity" with a concentration CaCO3 of greater than or equal to 50 mg per litre

The WFD had two default objectives regarding water quality. To prevent deterioration of the status of all surface water and groundwater bodies and to protect, enhance and restore all bodies of surface water and groundwater with the aim of achieving Good Status for surface water and groundwater by 2015.

The sampling for this investigation was undertaken in 2013 and 2014, near to the end of the period set out in the WFD. The River Avon is in good condition with regard to SRP according to the WFD but the River Stour and the estuary are in much poorer condition. This discrepancy between the water qualities of the two rivers has implications for Mudeford and Christchurch Harbour as a whole. This investigation has shown that the River Stour is the source of the majority of the nutrient flux into Christchurch Harbour, therefore reduction of the nutrient inputs into this river are of utmost importance if the water quality of Christchurch Harbour is to be improved which, at the time of sampling, was classified as 'moderate' with regard to SRP according to the WFD 50% of the time.

4.4.5 Conclusions

DOP concentrations varied on a weekly basis and seasonal variation in DOP concentration is evident at both the riverine and estuarine sites. DOP concentrations were higher in the River Stour than the River Avon, most likely due to differences in the base sediment of the two rivers, with the River Avon catchment composed of mainly chalk and the River Stour catchment composed of clay. DOP concentration was highest at Iford Bridge on the River Stour. This may be due to the point source inputs from a STW, which is located between the site at Throop and Iford Bridge. Increased fluxes of P into the estuary are associated with high river flow rates. In addition to this, hysteresis loops can be identified which indicate that phosphorus is associated with particles and flood events can lead to release of terrestrial P into river systems. SRP and TDP showed consistent non-conservative behaviour with regard to salinity and DOP showed non-conservative behaviour on some of the sampling dates, indicating fixation into phytoplankton and sedimentation in the estuary.

Results from this investigation indicated that DOP can be a significant fraction in lowland rivers and estuary systems and that as it has a bioavailable fraction, it needs to be incorporated into future eutrophication models. Legislation such as the EU water framework directive help drive reductions in inputs of macronutrients into riverine systems and this investigation has shown that there can be great variations in nutrient enrichment between two geographically close river catchment systems.

Chapter 5: Summary and conclusions

5.1 Synthesis of results

The overall goal of this research was to investigate how the concentration of both dissolved inorganic and organic forms of phosphorus vary seasonally and with extreme changes in river flow in the lower reaches of two rivers and the receiving waters of an estuary. To achieve this aim, a novel method to determine DOP concentration in natural water samples was developed as reported in chapter three of the thesis. The combination of using ICP-MS to measure TDP and the established colourimetric method of measuring SRP provides reliable estimates of DOP in both river and estuarine water samples. A novel, high frequency set of measurements of DOP were made over an annual period at sites in the lower reaches of the Hampshire Avon river, Dorset Stour river and Christchurch Harbour estuary. Additionally, seasonal changes in TDP, SRP and DOP concentrations were compared with a large core set of water quality measurements made at each of the sites over the annual period of monitoring. The impact of changing river flow on the flux of inorganic and organic dissolved phosphorus has been investigated and it has been shown that periods of high river flow are responsible the majority of the annual flux.

The first part of this thesis describes inorganic nutrient concentrations over a year at four sites, 3 riverine and one estuarine, as part of a core set of data analyses. A weekly sampling programme of the four sites was undertaken from April 2014 to August 2014. This part of the investigation used techniques which are well established in the literature for the analysis of macronutrients (nitrate, phosphate and silicate) and the deployment of multiparameter sondes allowed other water quality parameters (temperature, conductivity/salinity, turbidity, pH, dissolved oxygen saturation, chlorophyll α concentration) to be determined.

The second part was to develop a technique to determine DOP concentrations in stored water samples using a novel technique of analysing total phosphorus concentration (ICP-MS) combined with a well-documented technique of analysing inorganic phosphorus (colourimetry). This is important because previous studies investigating DOP use a variety of different techniques to analyse total phosphorus, but most use the same method to analyse the inorganic phosphorus fraction (Table 1). These methods include addition of an oxidising agent, enzymatic hydrolysis and high temperature combustion as well as other methods such as ultraviolet degradation. Each of these methods has a different efficiency in converting organic P molecules to their inorganic form, depending on the P molecules present, and may underestimate the total phosphorus concentration in the sample (Ormaza-González and Statham, 1996). The problem with the lack of a standardised method for the analysis of total phosphorus is that future water quality legislation requires accurate concentrations of nutrients in order to tackle eutrophication and other nutrient related issues (Ferreira et al., 2011).

The method developed in this research project provides an alternative technique, which does not require the conversion of organic and colloidal forms of phosphorus to inorganic phosphate but measures the total phosphorus using mass spectrometry after ionisation in argon plasma. Analyses of a certified reference material containing a known concentration of total phosphorus, and both refractory and labile organic phosphorus molecules indicate that ICP-MS is a robust technique for the analysis of dissolved phosphorus. In addition to this, when used in conjunction with analysis of SRP, the concentration of DOP can be reliably determined.

The third part was to analyse dissolved organic phosphorus in the same system using the novel combination of ICP-MS and spectrophotometry developed in this investigation. The current study proposes that ICP-MS should be used as a method to analyse TDP as over 95% of the TDP was detected using this method. At the current time, the most accepted method for analysing SRP is the molybdenum blue assay (Murphy and Riley, 1962) and the combination of these two techniques can provide a reliable DOP concentration.

5.2 Future perspectives.

While the methods applied in this study have been shown to be successful in providing a consistent DOP concentration in environmental water samples, there are additional data analyses, which would benefit nutrient risk assessment models.

While ICP-MS analysis can determine the quantity of DOP in a sample, it does not provide information on the organic compounds present. Organic phosphorus refers to a diverse group of chemical compounds so the broad term DOP can contain both labile and recalcitrant molecules. Techniques such as Phosphorus-31 nuclear magnetic resonance spectroscopy show promise in determining the composition of the organic matter in water sample (Baldwin, 2013) and when used in conjunction with other quantitative techniques, as described in the current study, may provide a more detailed picture of DOP speciation and dynamics.

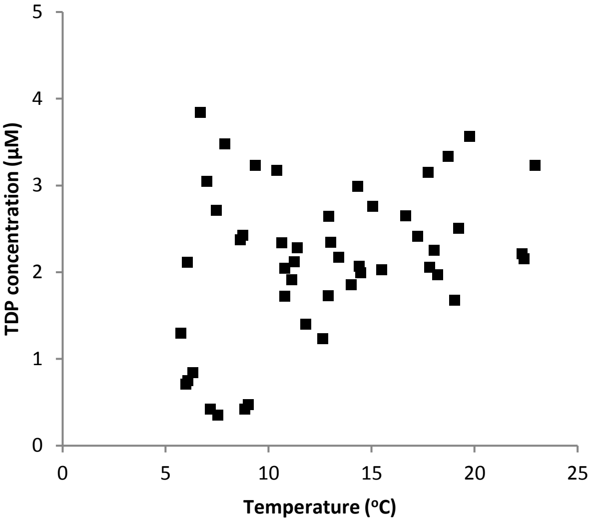
This study has also highlighted the importance in the frequency of sampling in the identification of short-term fluctuations in nutrient concentration in riverine systems. This current study sampled on a weekly basis, however, other studies are investigating phosphorus using high resolution, in situ nutrient autoanalysers (Yücel et al., 2015; Bowes et al., 2015). These studies provide insight into nutrient concentrations at a site for an extended time period at very high resolution, helping to identify changes in nutrient concentration accurately and quickly. Although these high-resolution sensors are very useful in quantifying nutrients, they are restricted in terms of analysis. For example, filtration of a sample is difficult as filters can become clogged with particulate matter. Additionally, oxidation of organic matter is difficult to achieve and relies on ultraviolet light, which may not be the most efficient method. Therefore, at the current time, there is a balance when analysing nutrients between obtaining high frequency, but low precision data or higher precision data but analysed at a lower frequency. However, the development of ‘lab on a chip’ sensors is promising for future analysis of inorganic and organic nutrients at high resolution.

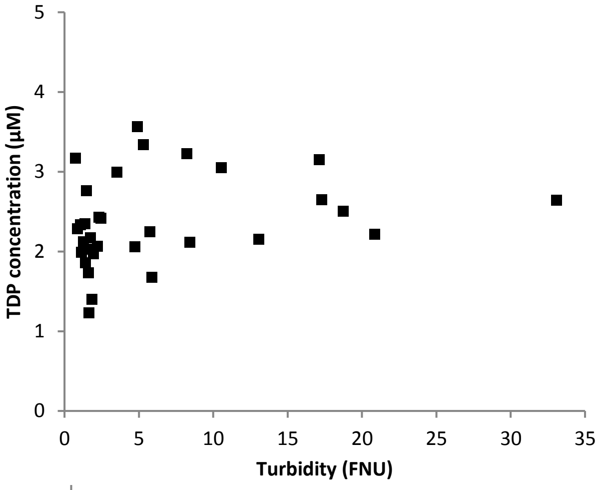
Further research is needed into determining the nature of organic phosphorus, because it represents a substantial pool of phosphorus in aquatic and marine ecosystems. If government targets to reduce phosphorus delivery to coastal waters are to be met, routine measurements of DOP need to be considered.

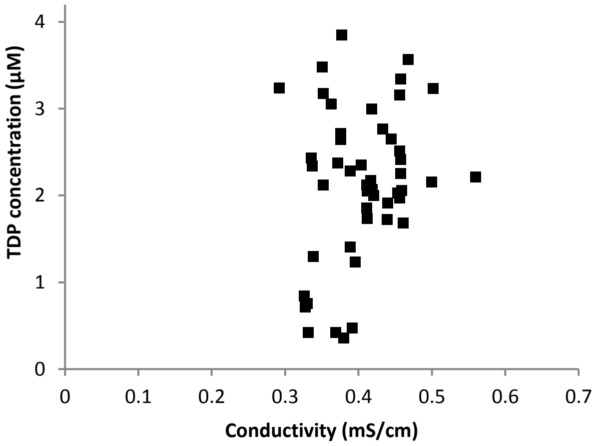
Appendix 1 – Relationships between phosphorus fractions and environmental parameters

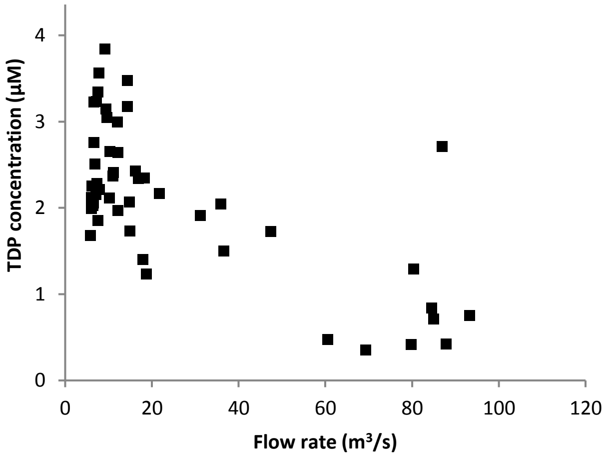
1.1 Knapp Mill

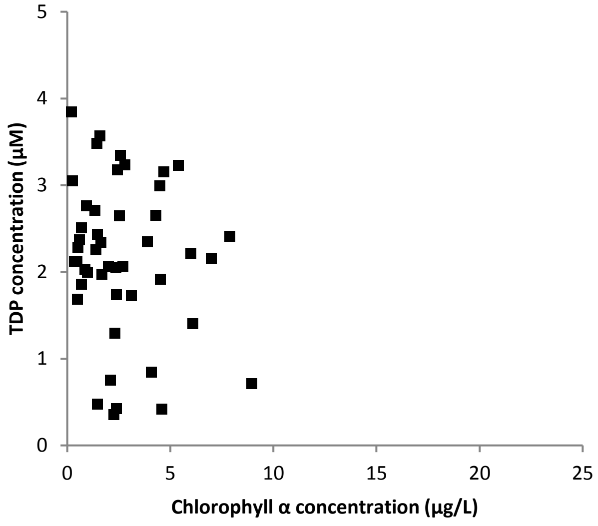
1.1.1 Total Dissolved Phosphorus (TDP)

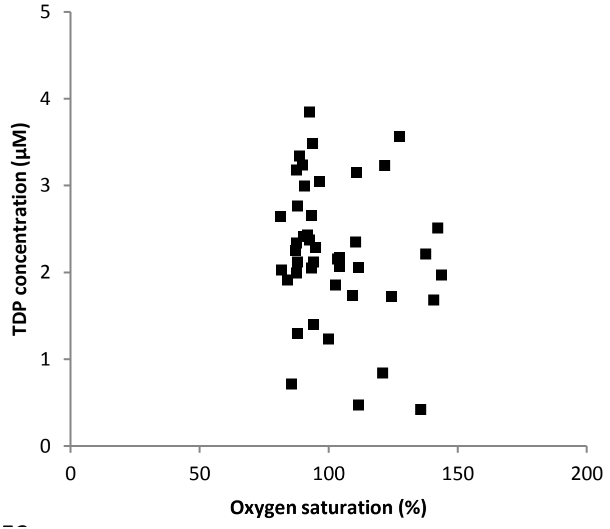




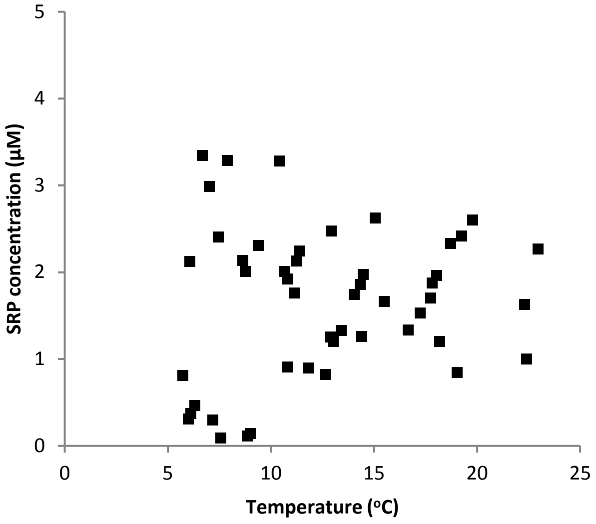


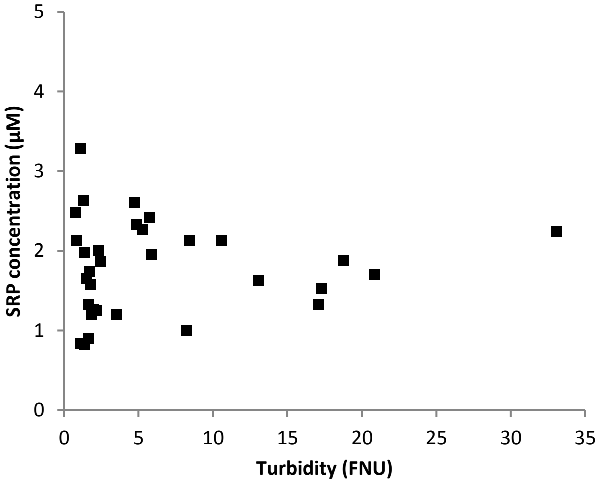


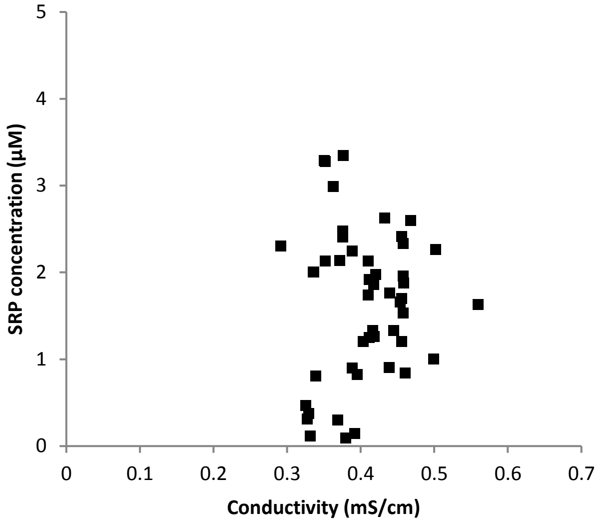


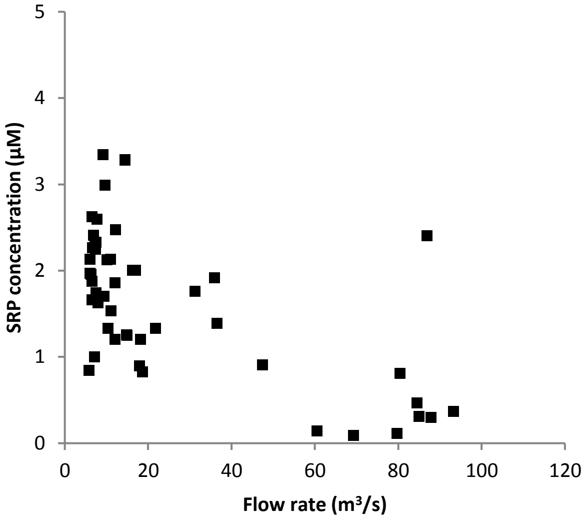


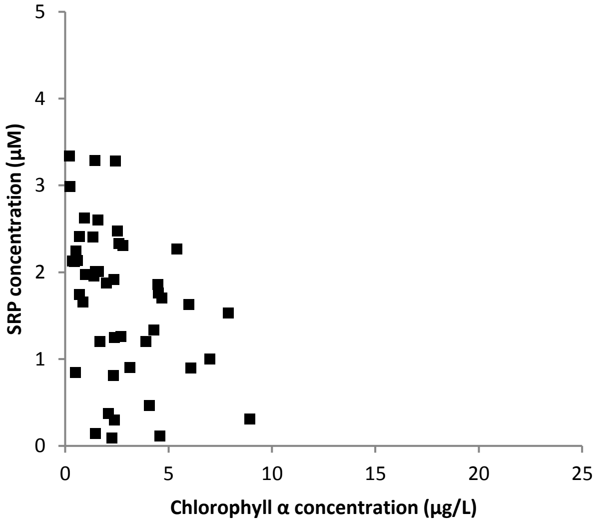
1.1.2 Soluble reactive phosphorus (SRP)

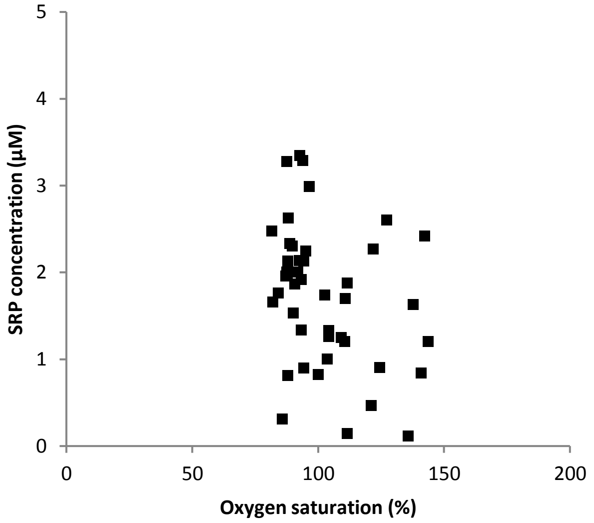




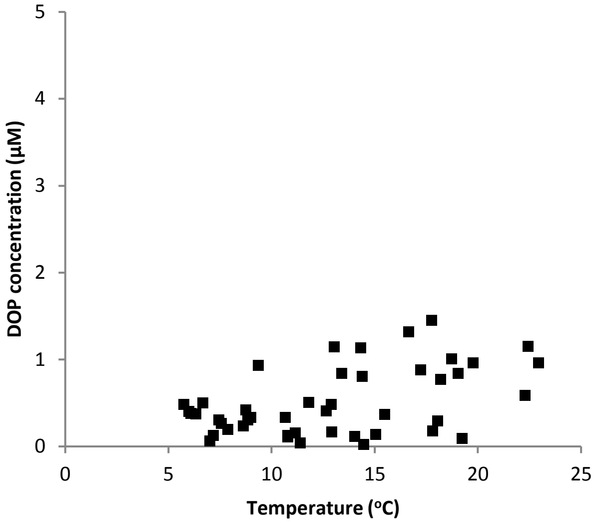


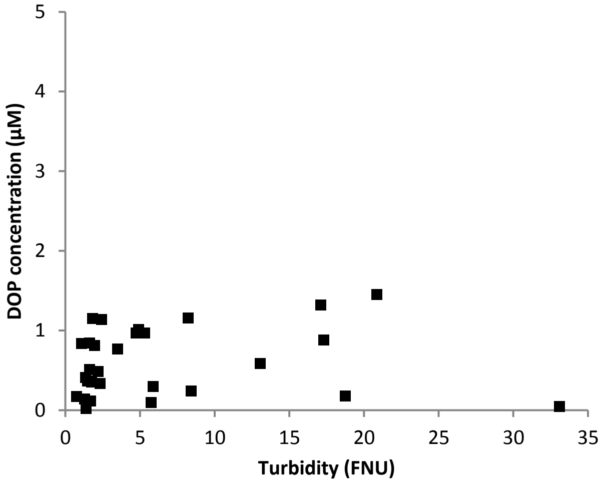


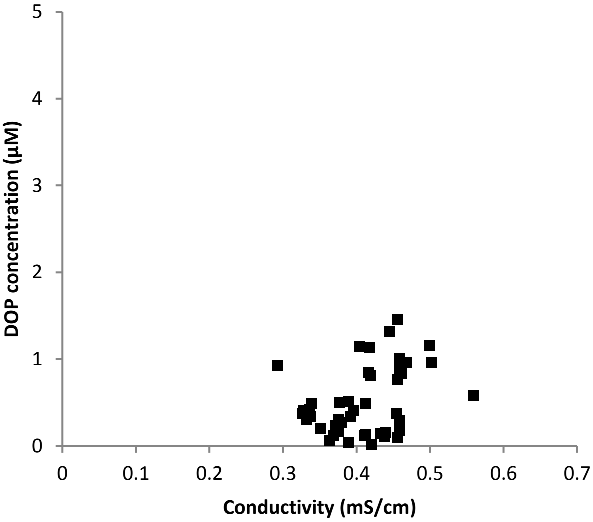


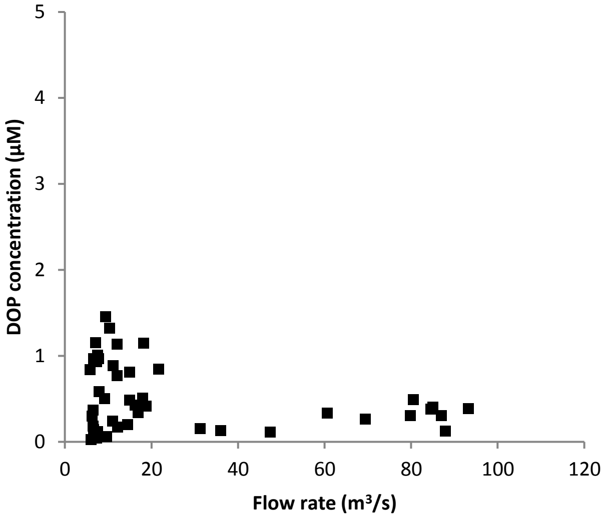


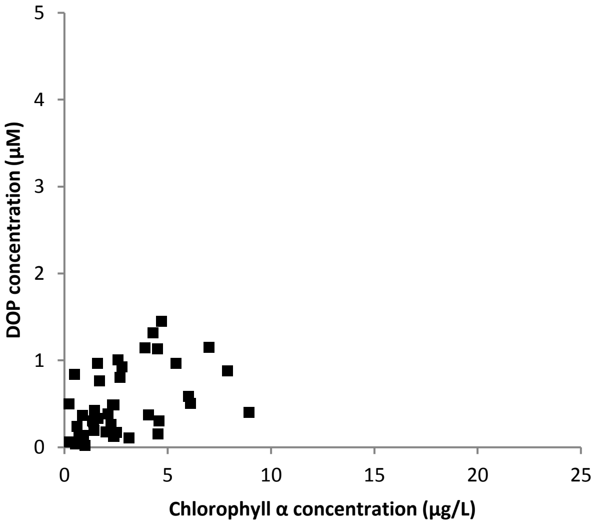
1.1.3 Dissolved organic phosphorus concentration (DOP)

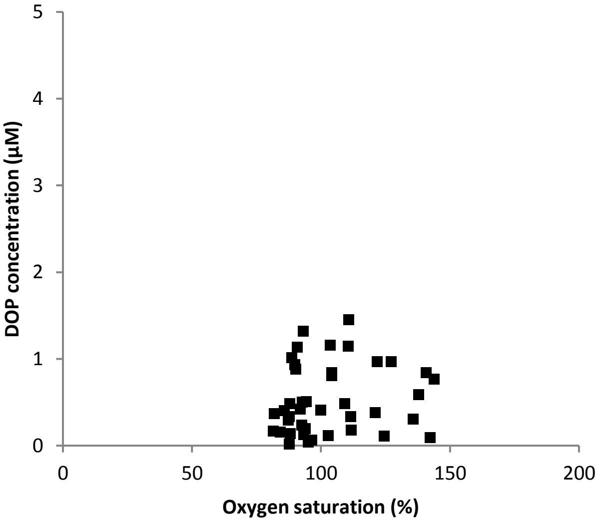




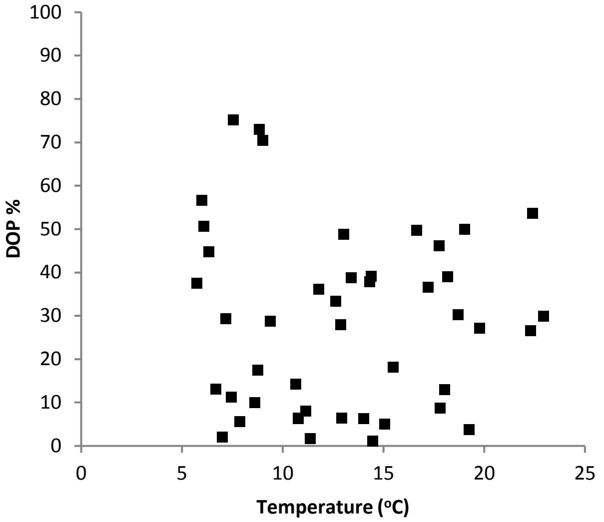


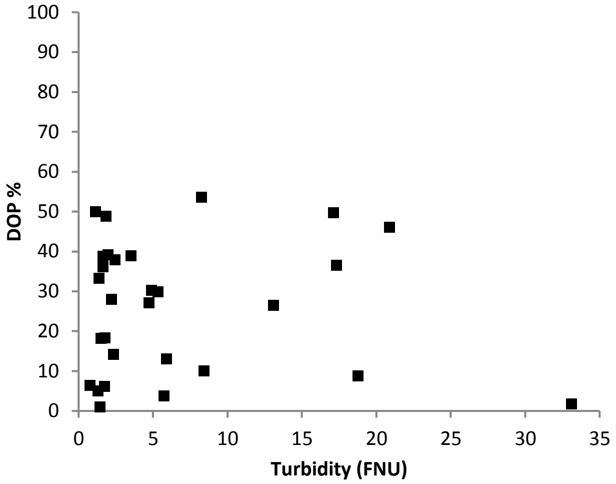


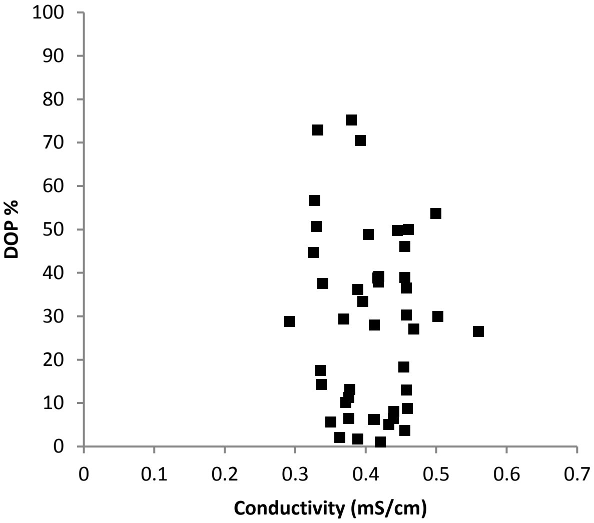


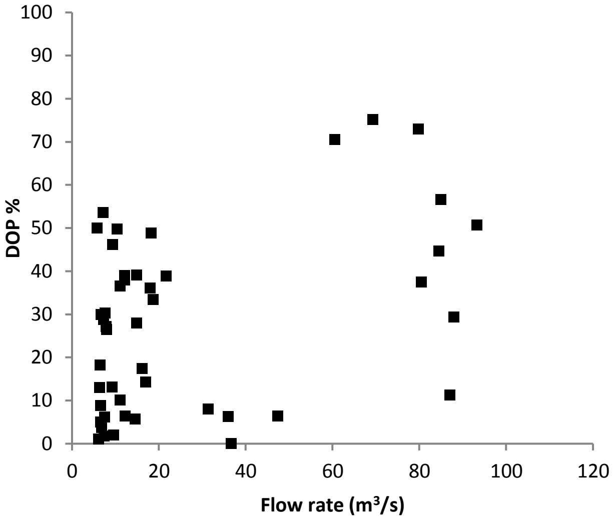


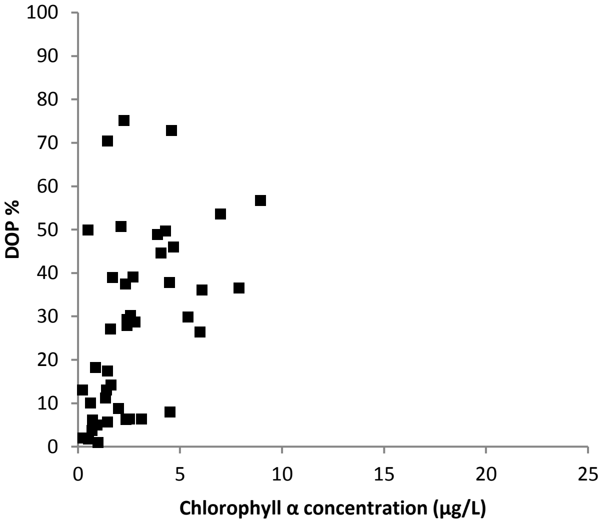
1.1.4 Dissolved organic phosphorus %

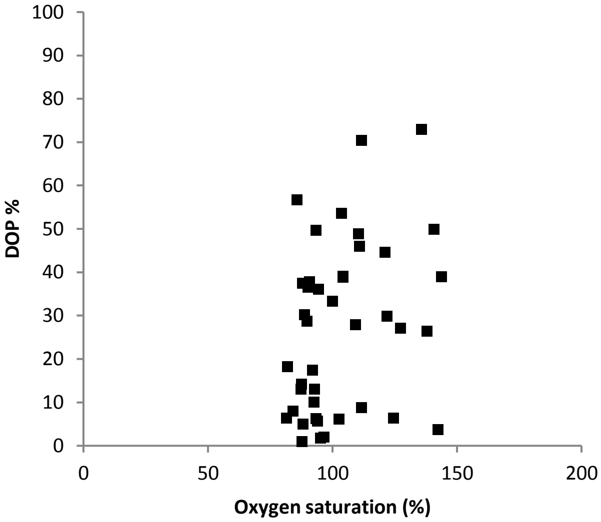






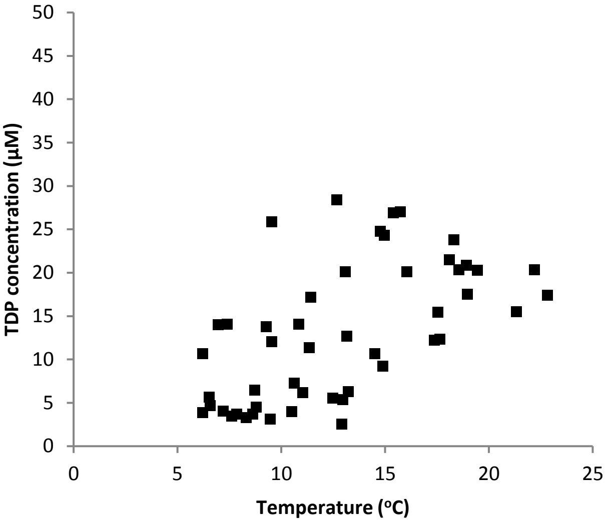


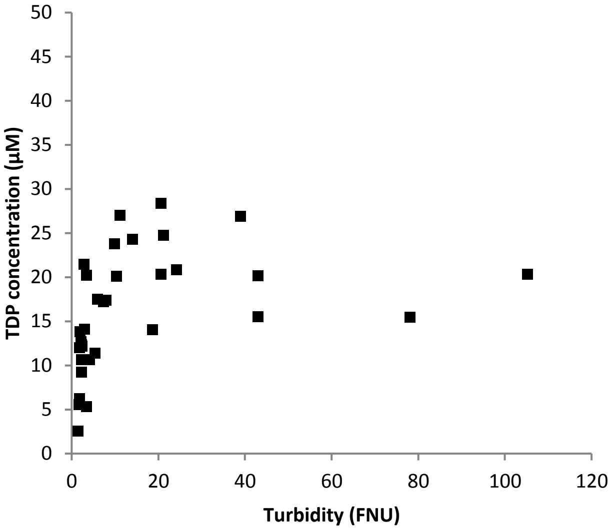


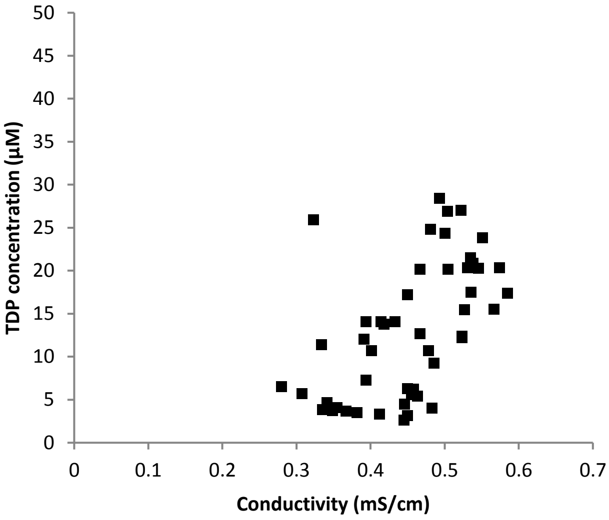


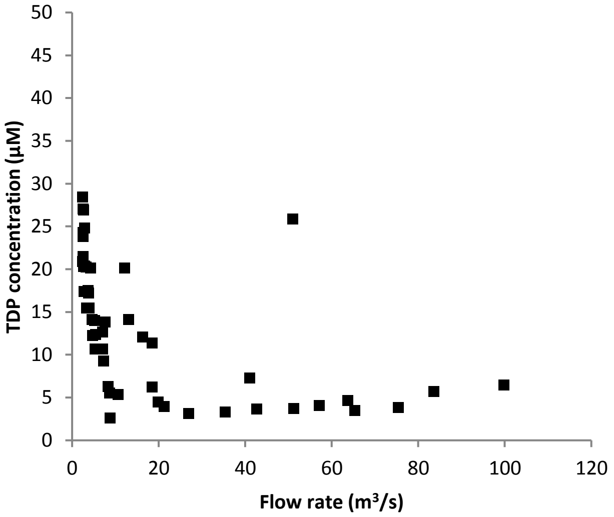
1.2 Throop

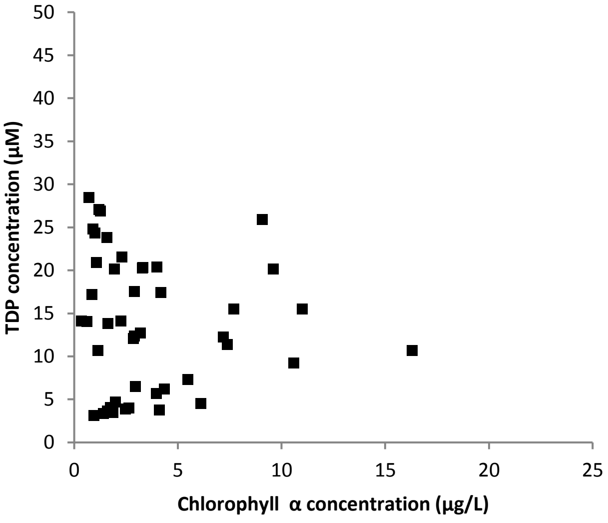
1.2.1 Total Dissolved Phosphorus

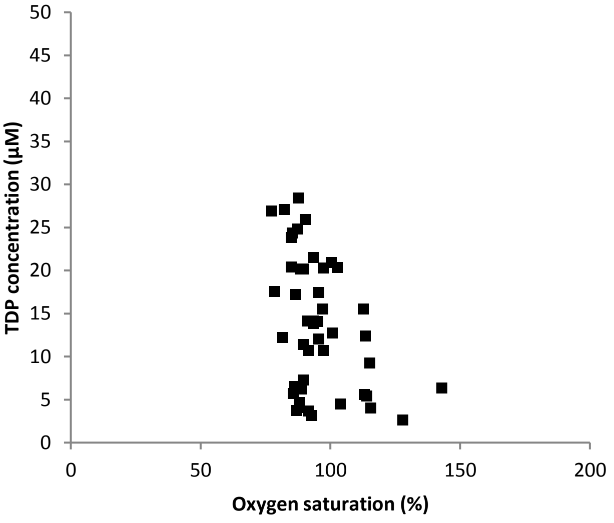




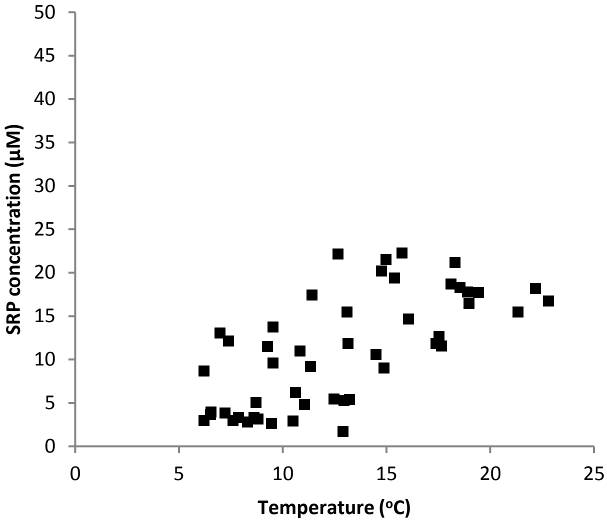


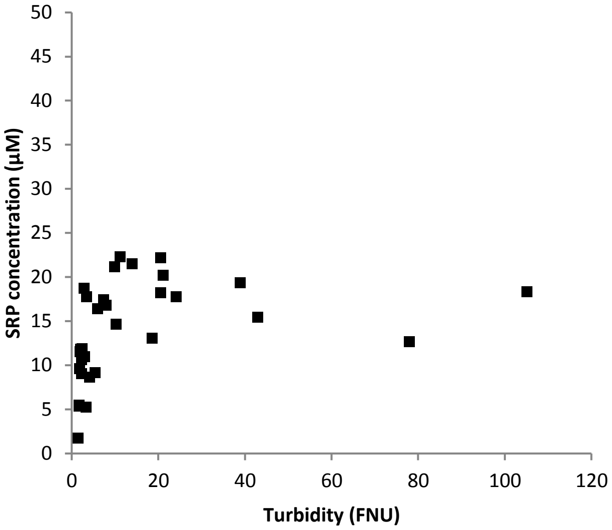


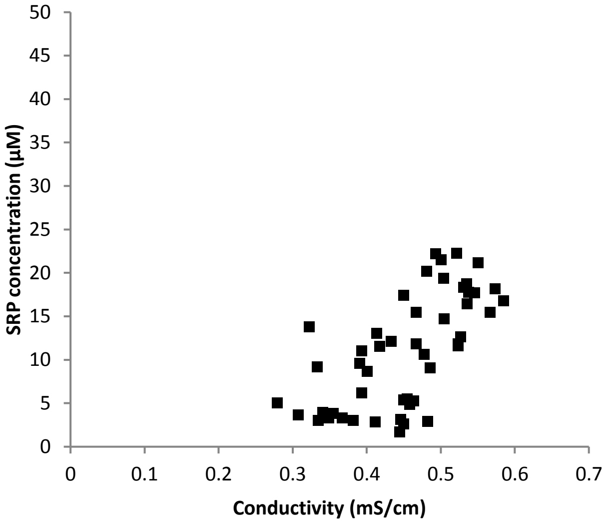


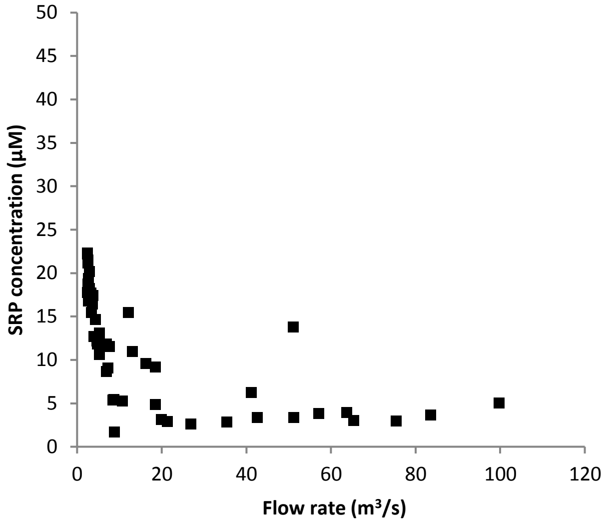


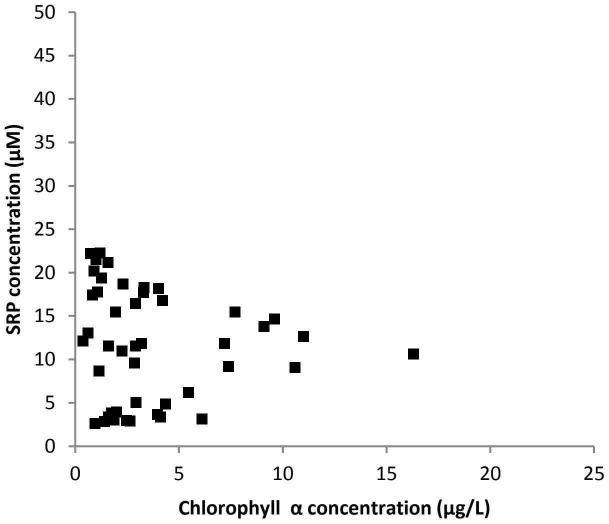
1.2.2 Soluble reactive phosphorus (SRP)

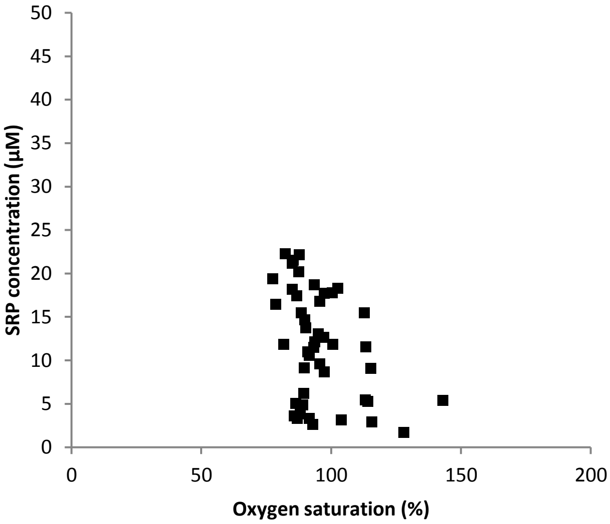




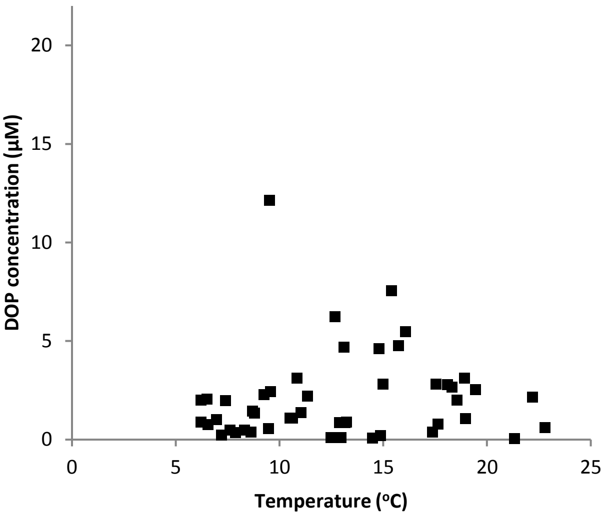


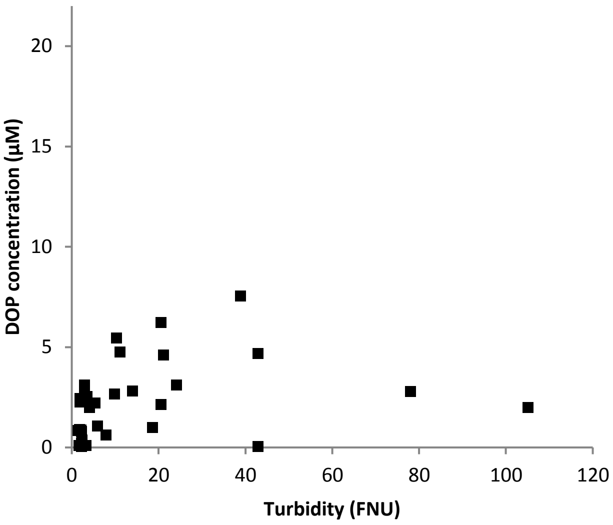


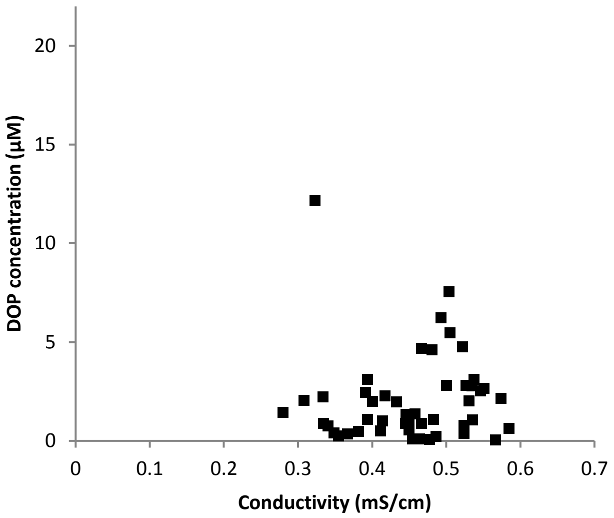


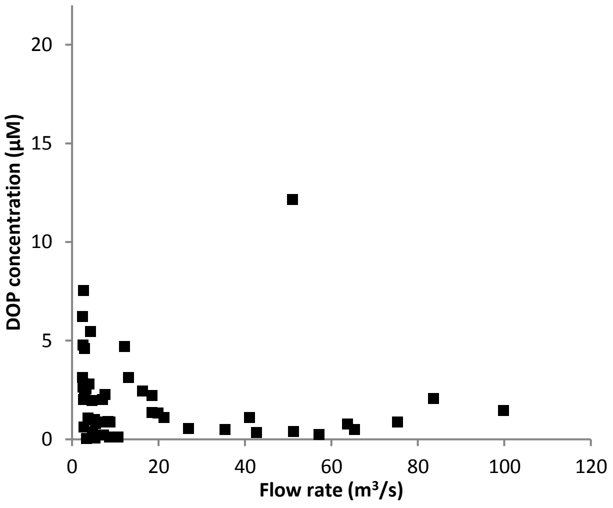


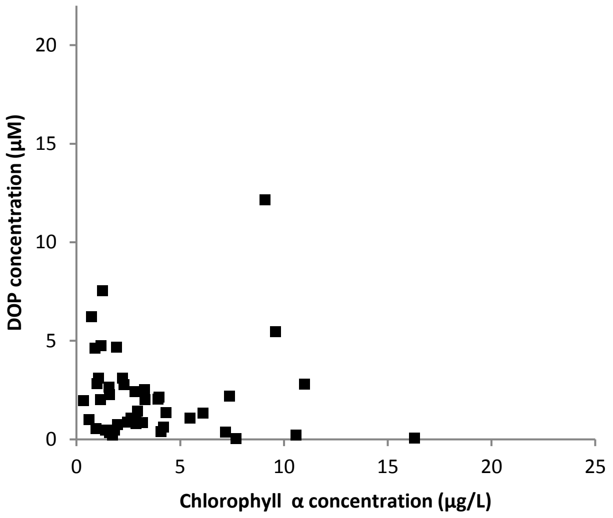
1.2.3 Dissolved organic phosphorus

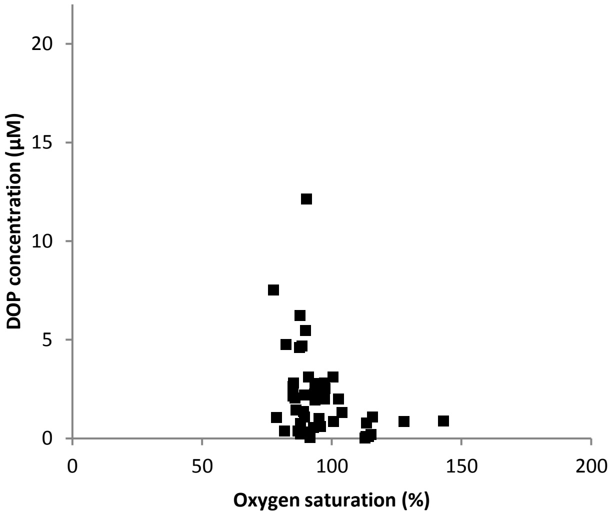




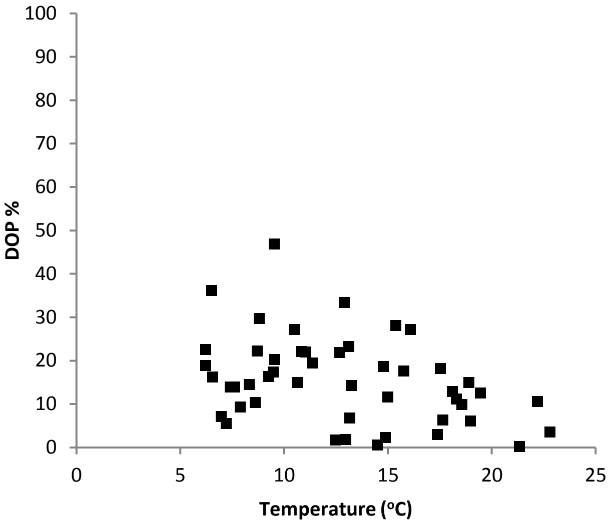


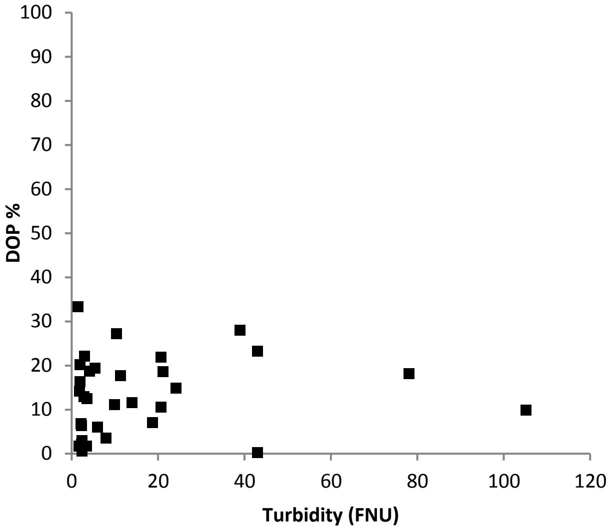


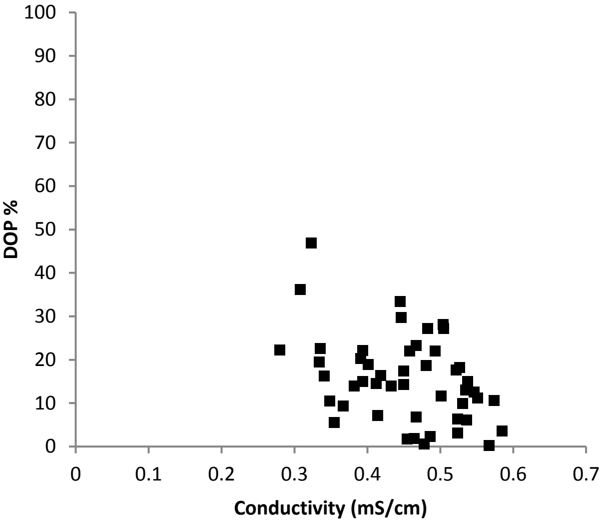


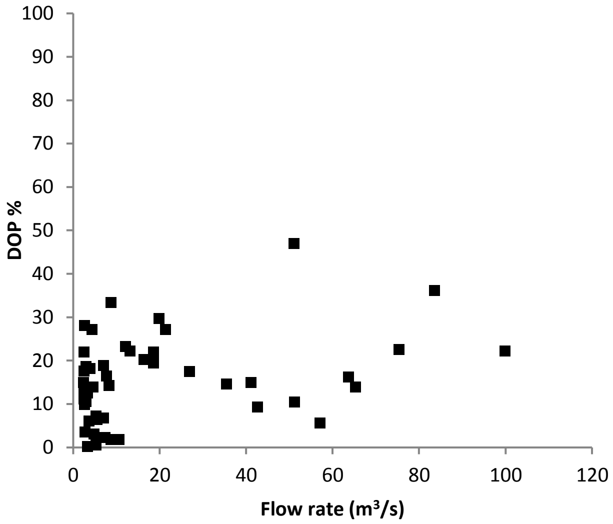


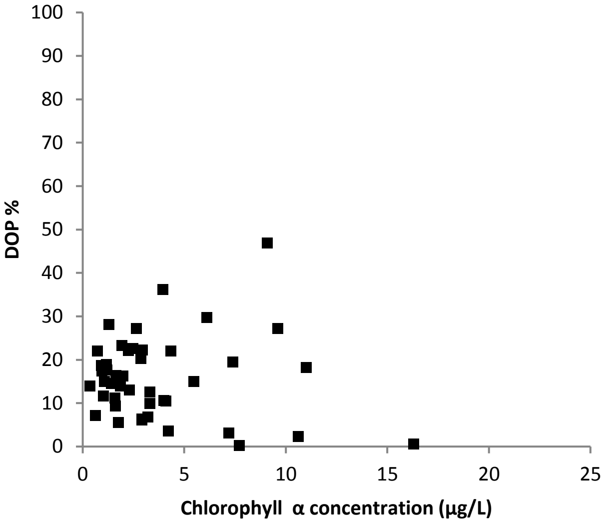
1.2.4 Dissolved organic phosphorus %

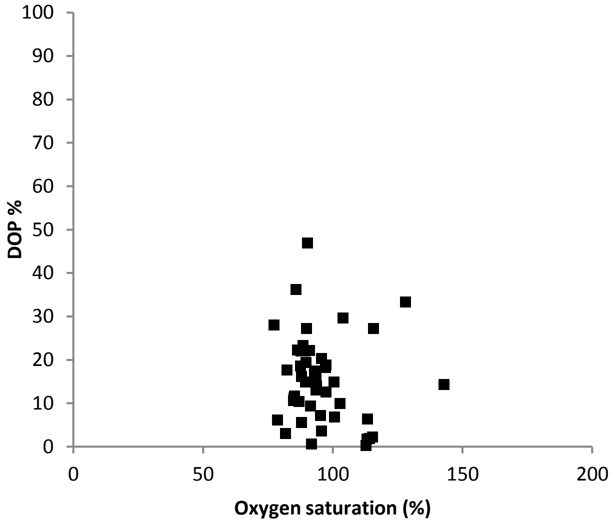






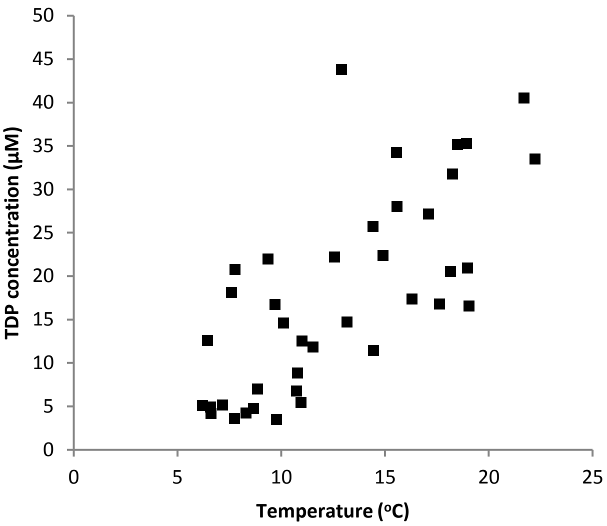


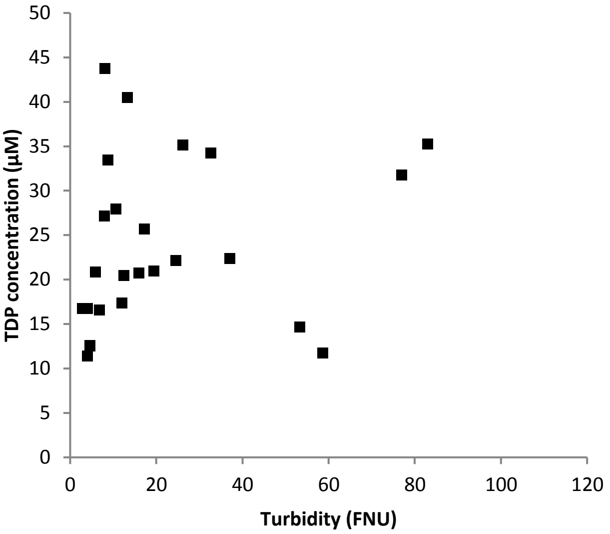


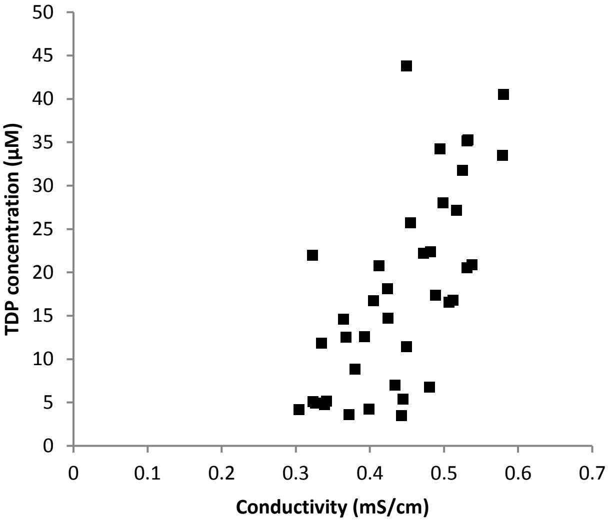


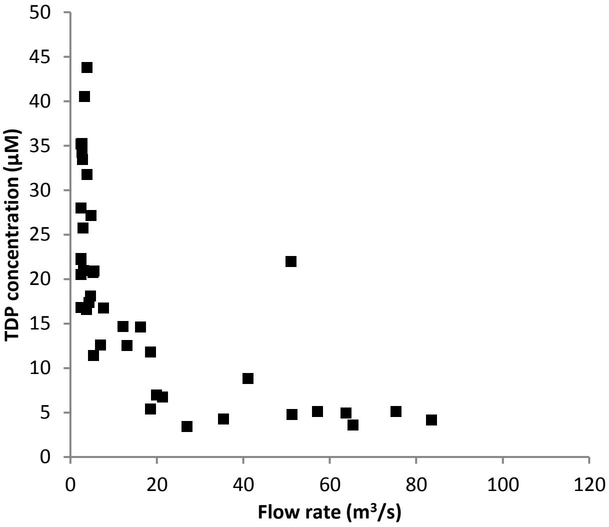
1.3 Iford

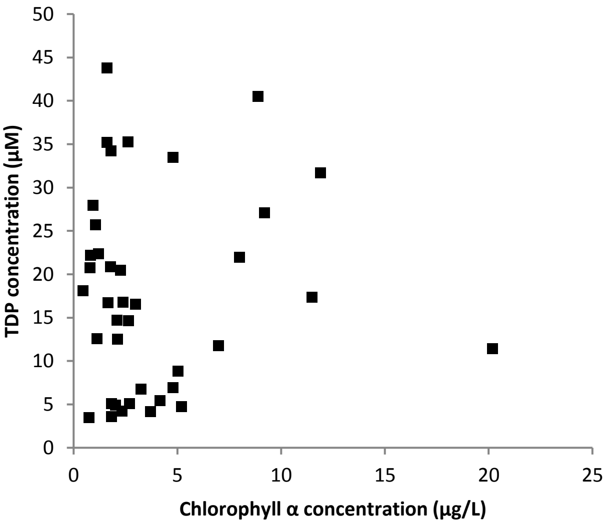
1.3.1 Total dissolved phosphorus (DTP)

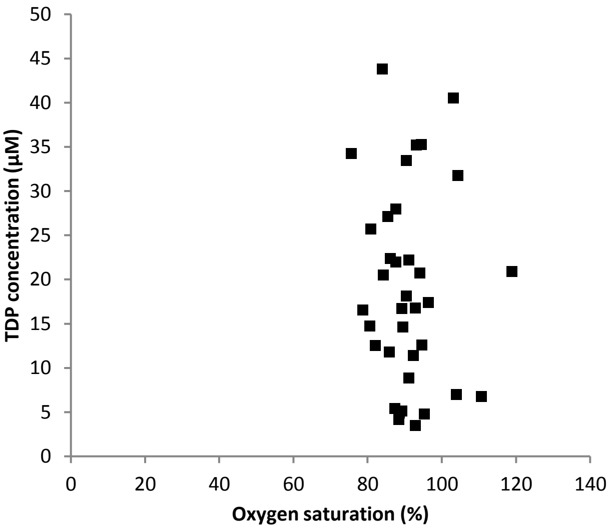




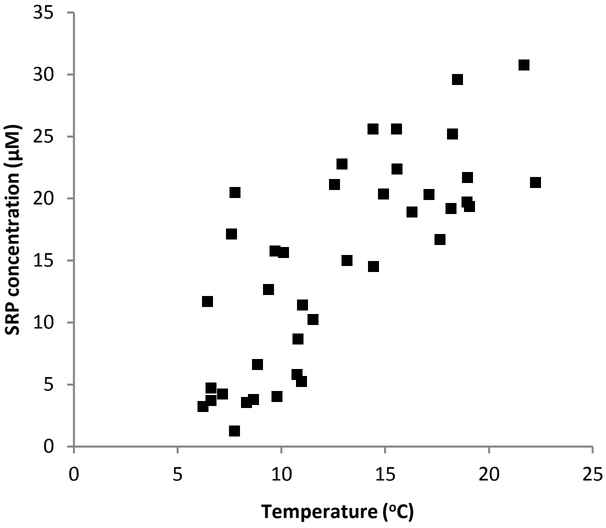


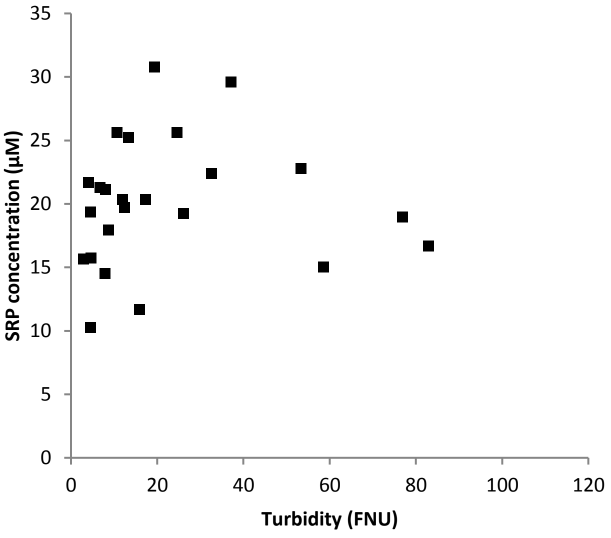


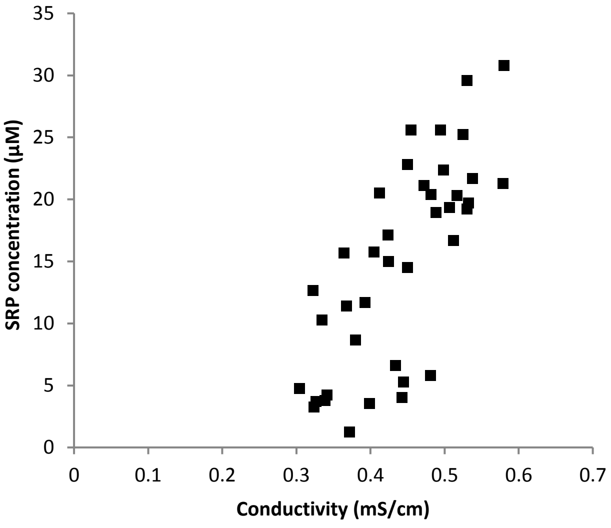


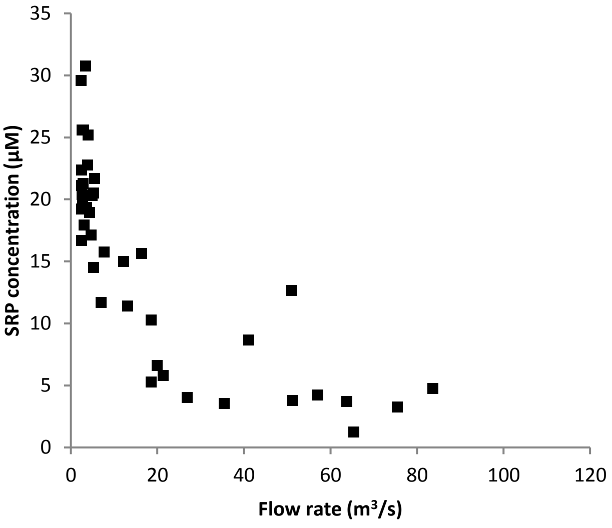


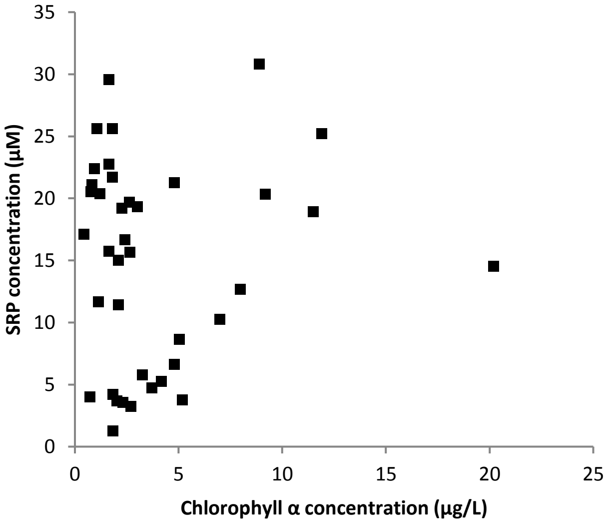
1.3.2 Soluble reactive phosphorus (SRP)

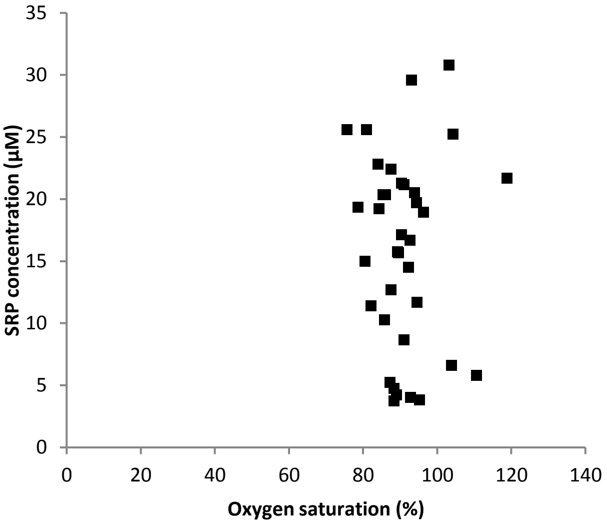




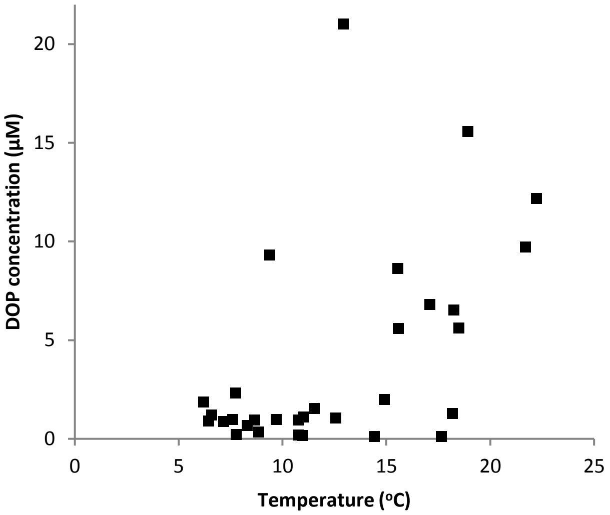


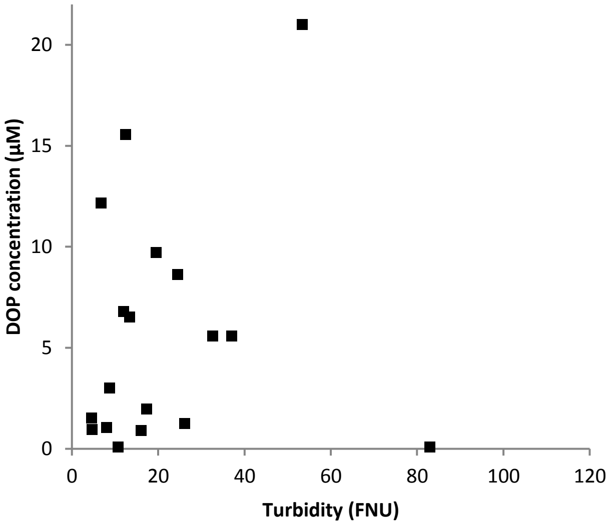


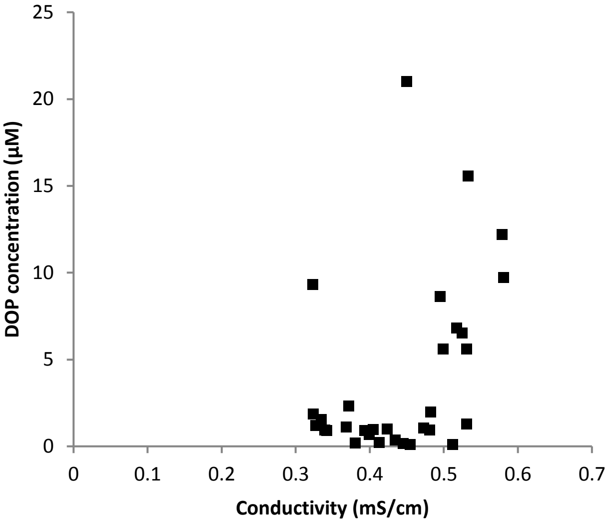


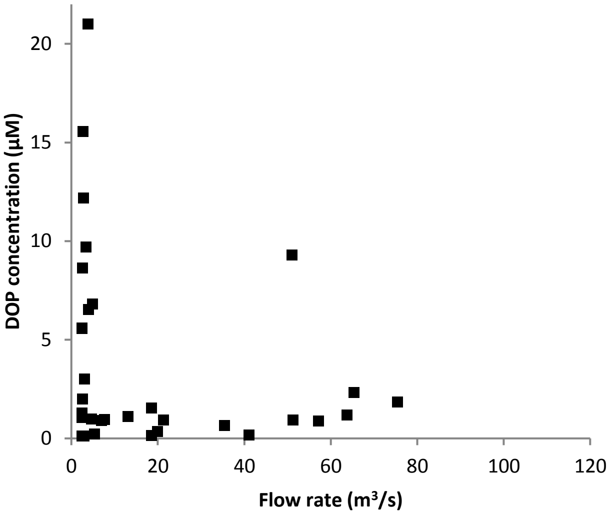


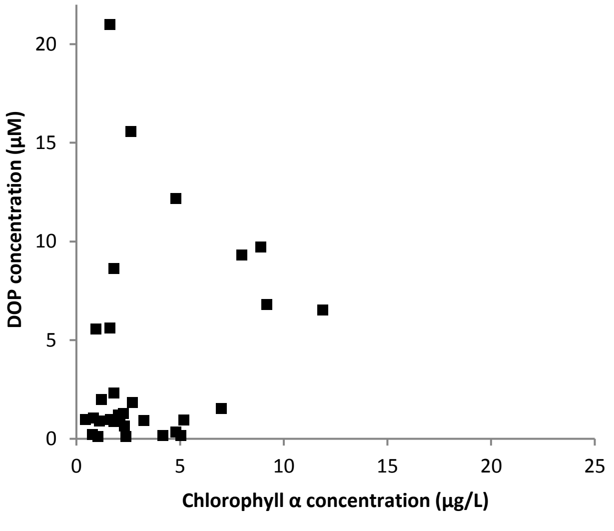
1.3.3 Dissolved organic phosphorus (DOP)

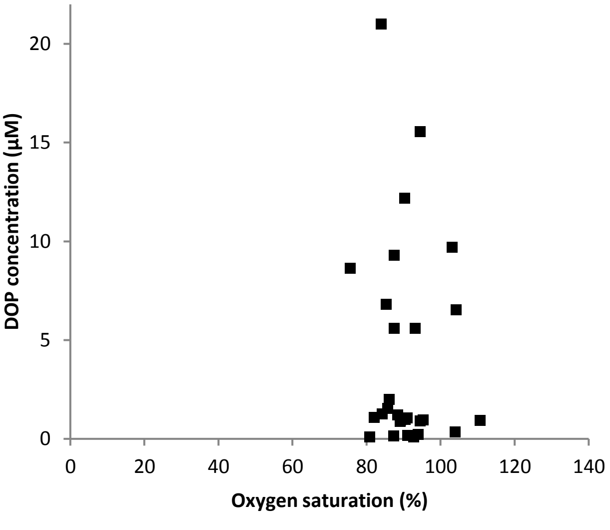




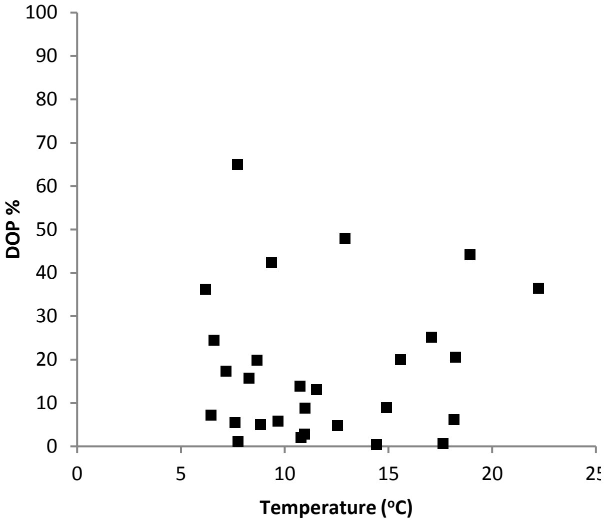


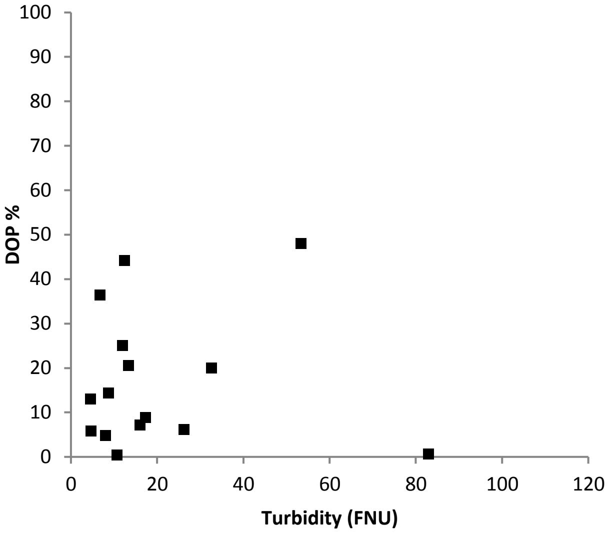


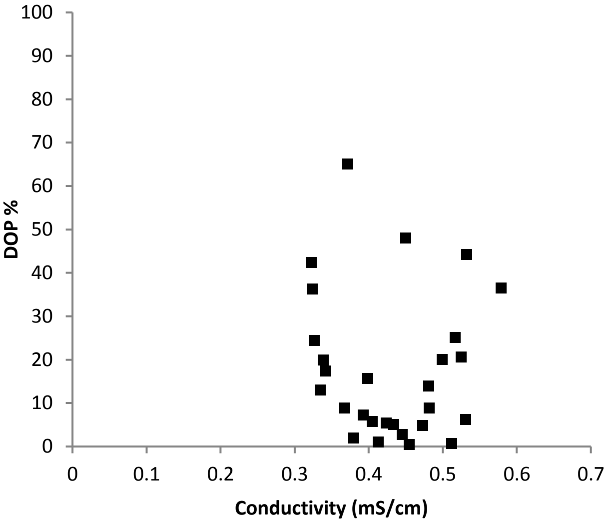


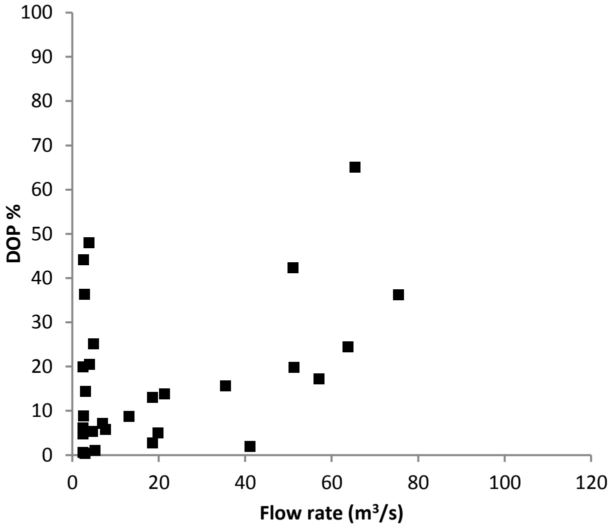


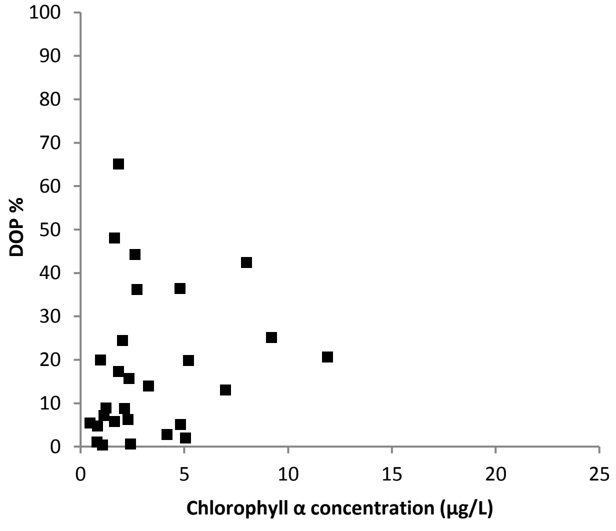
1.3.4 Dissolved organic phosphorus %

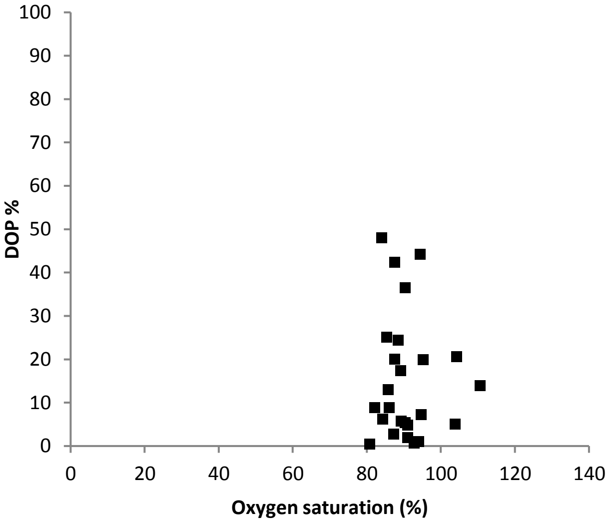






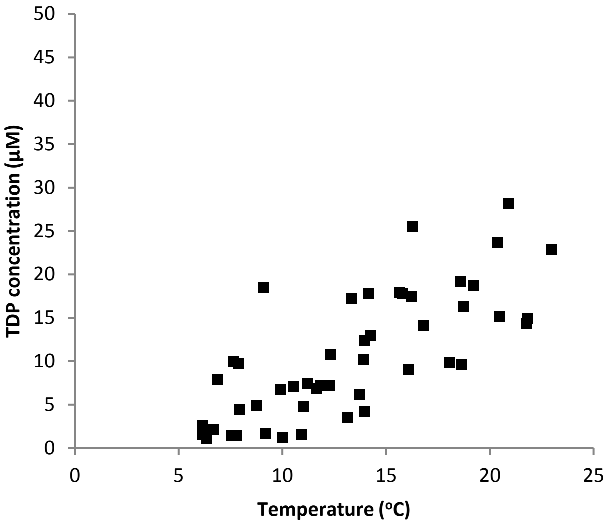


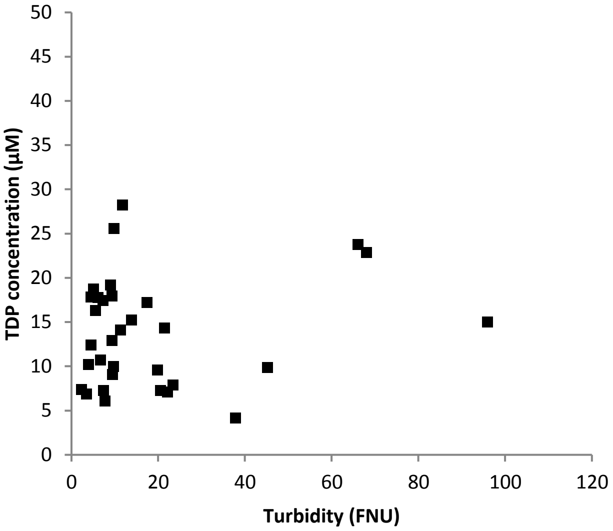


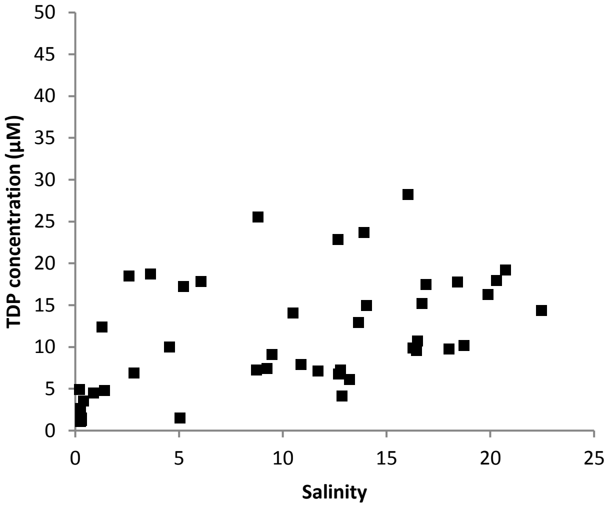


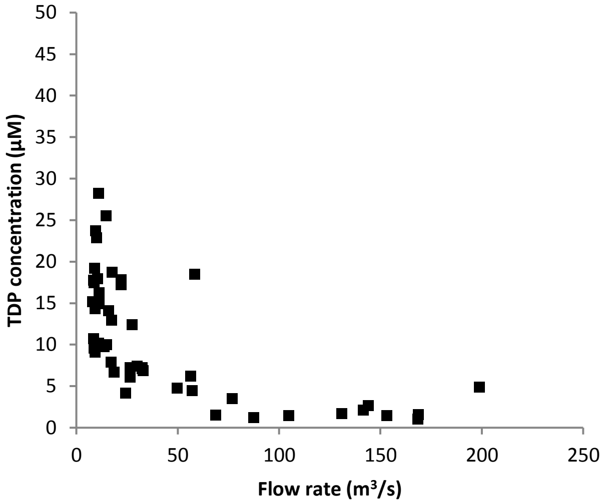
1.4 Mudeford Quay

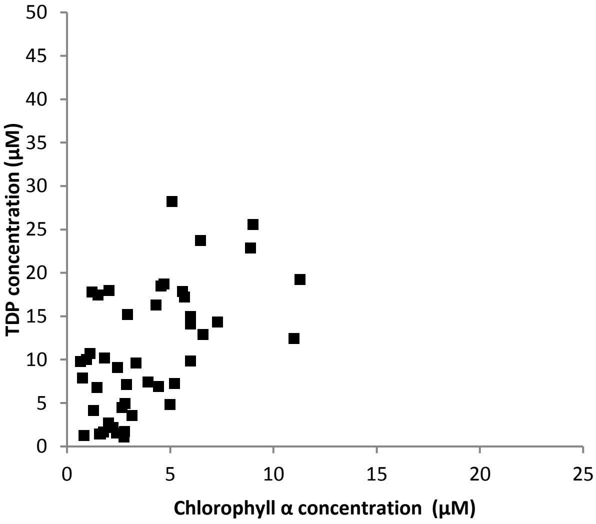
1.4.1 Total dissolved phosphorus (TDP)

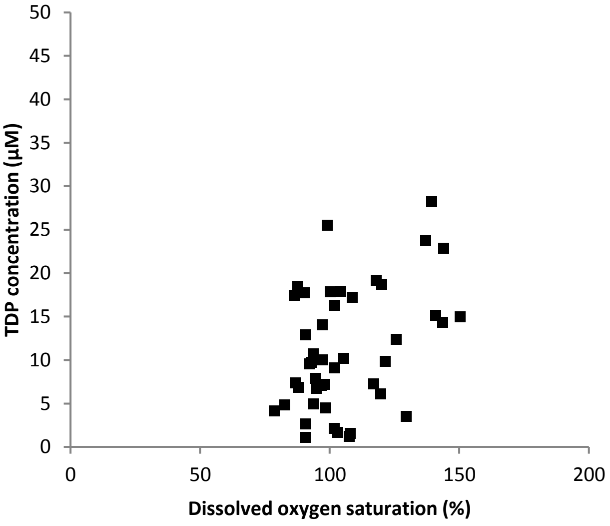




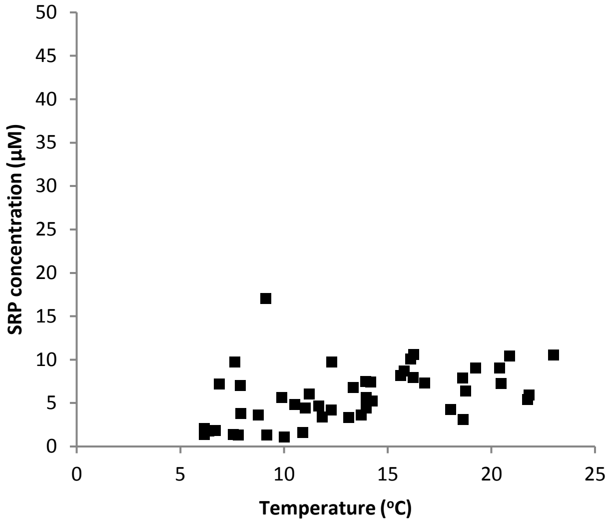


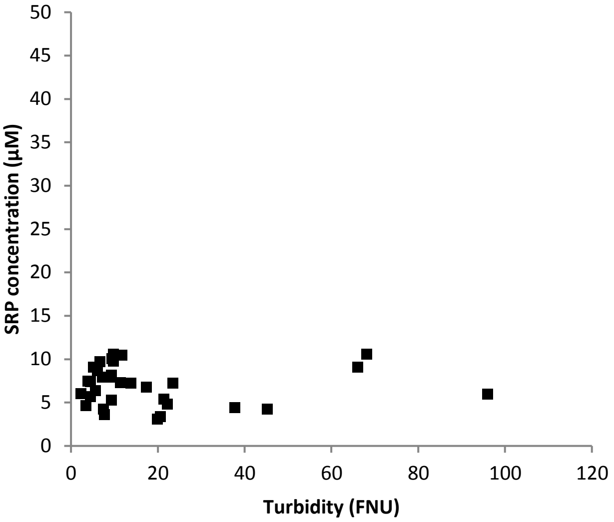


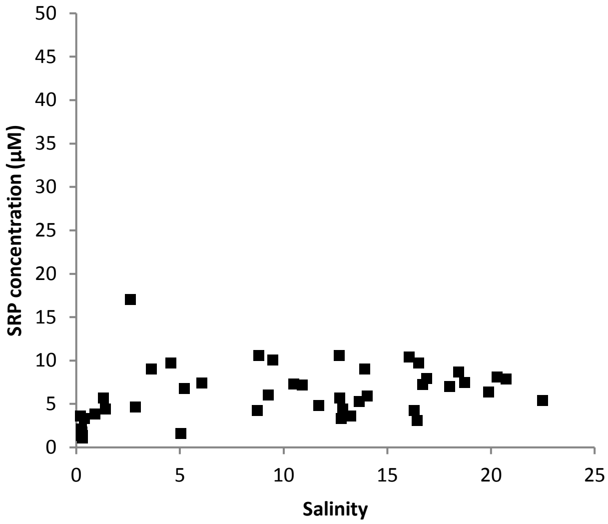


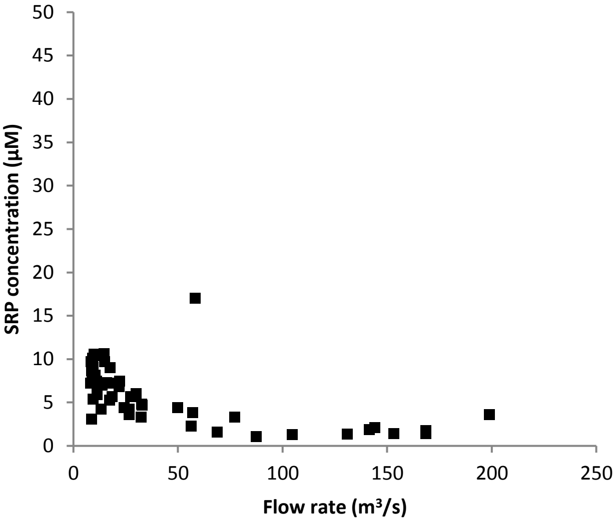


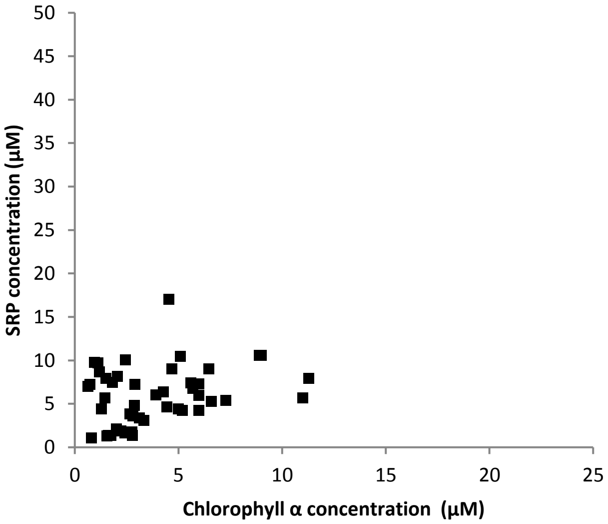
1.4.2 Soluble reactive phosphorus (SRP)

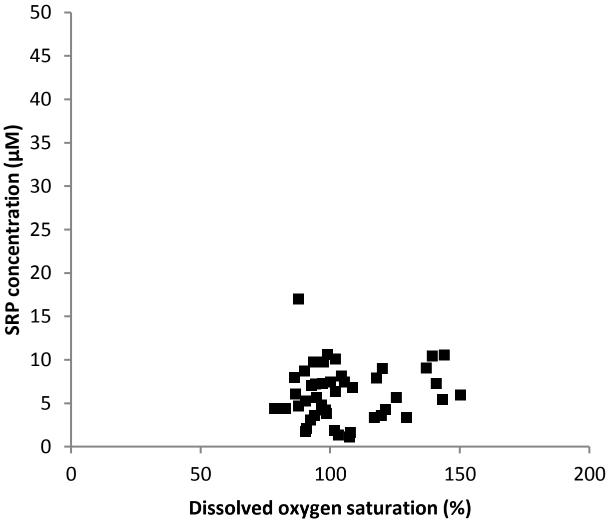




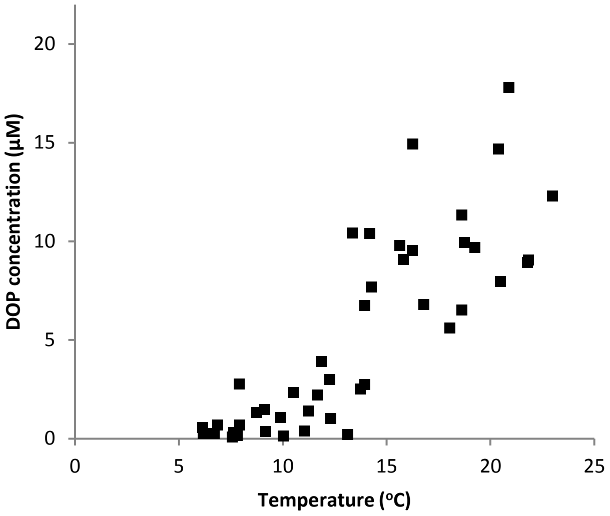


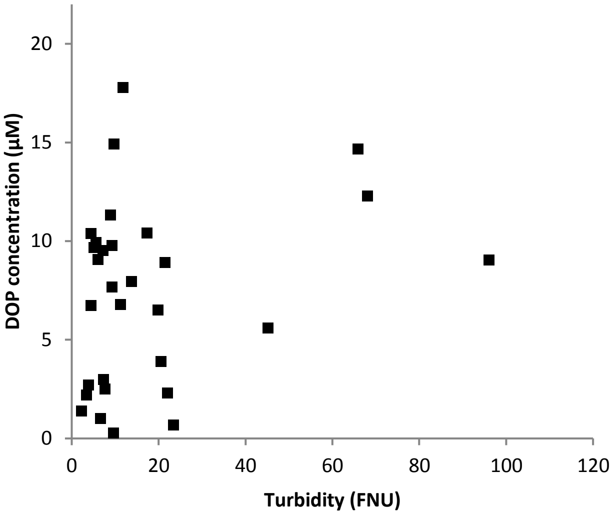


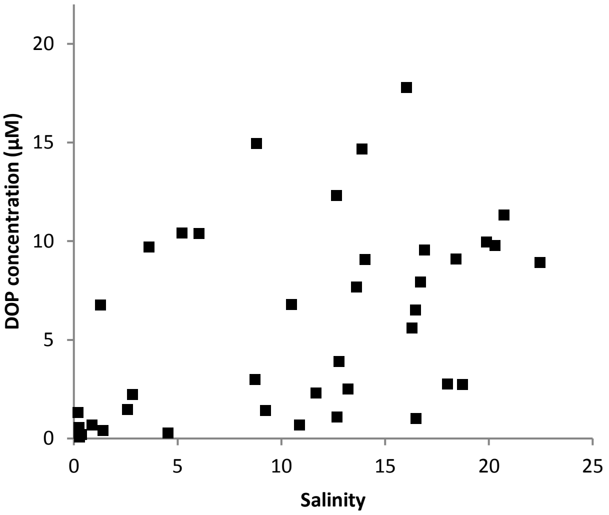


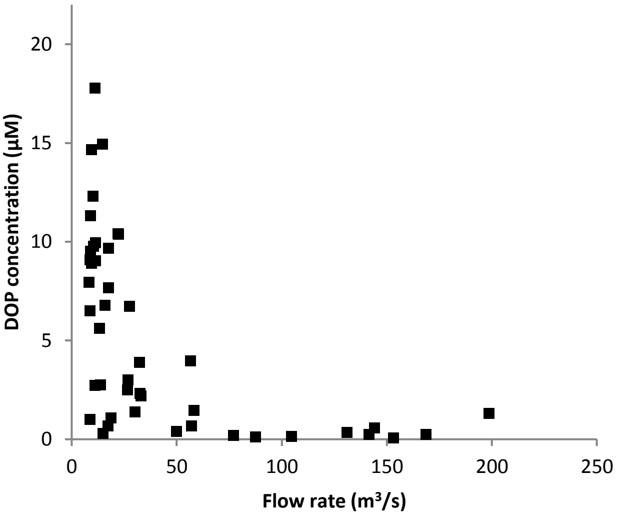


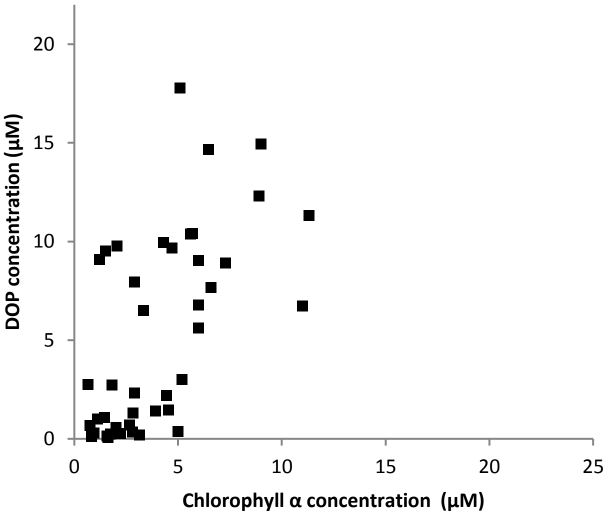
1.4.3 Dissolved organic phosphorus (DOP)

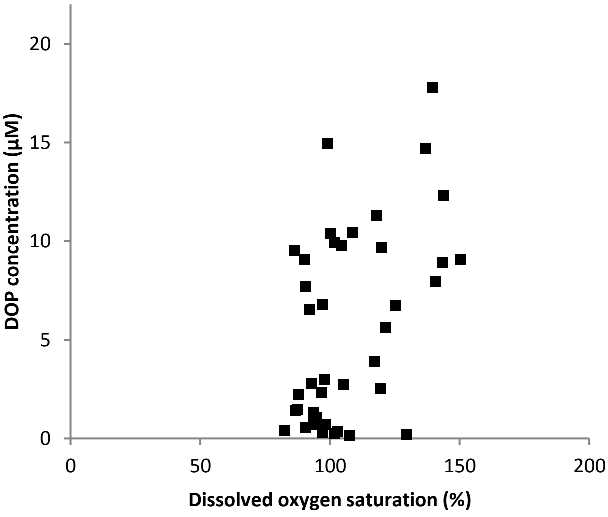




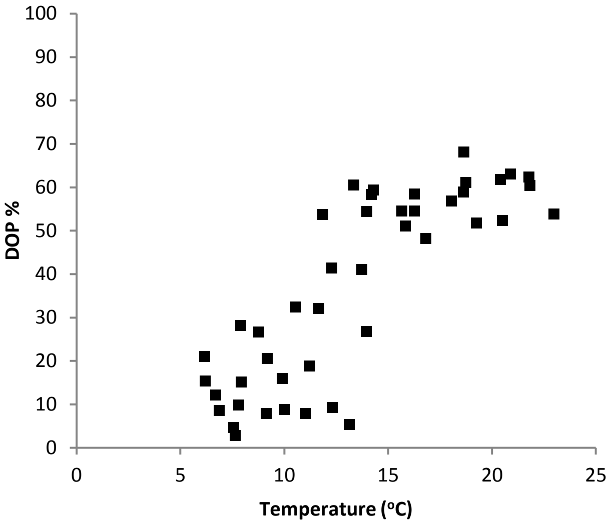


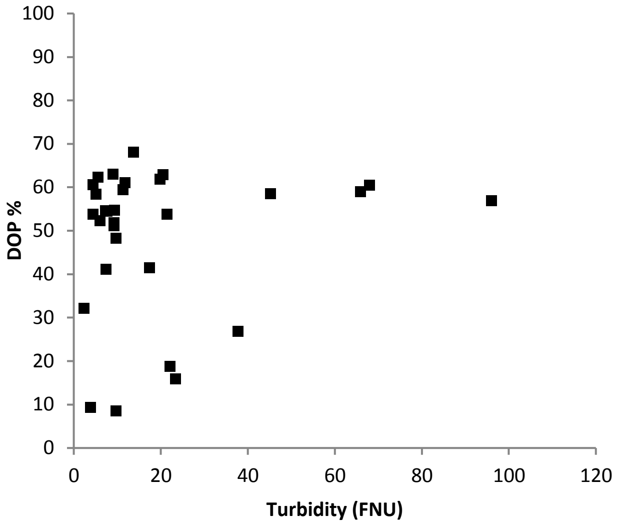


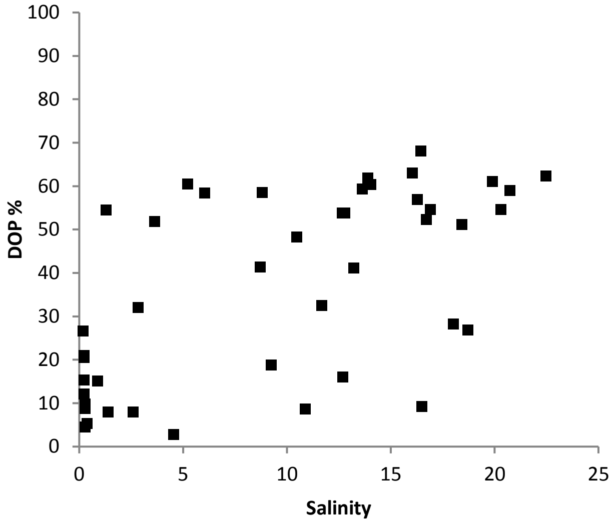


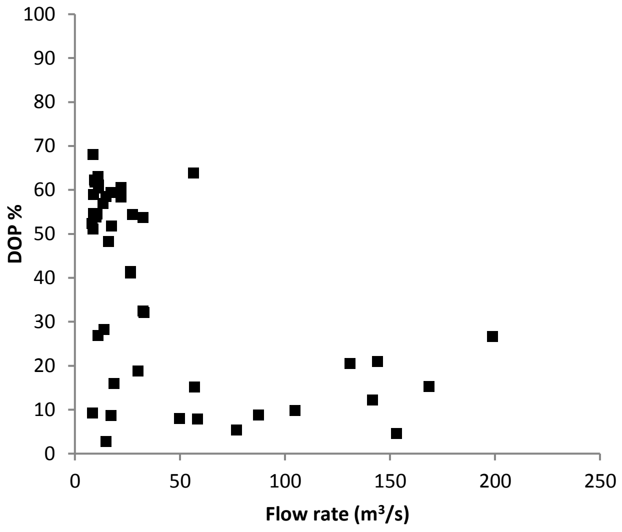


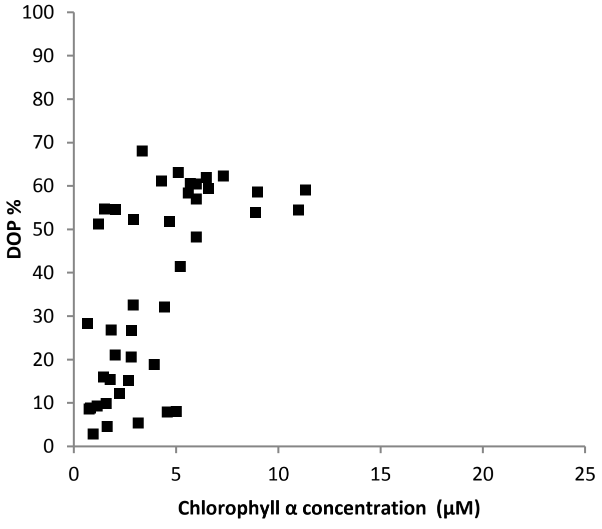
1.4.4 Dissolved organic phosphorus %

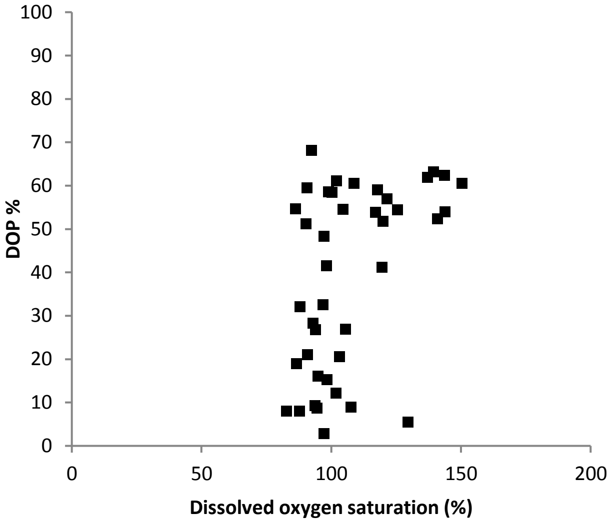








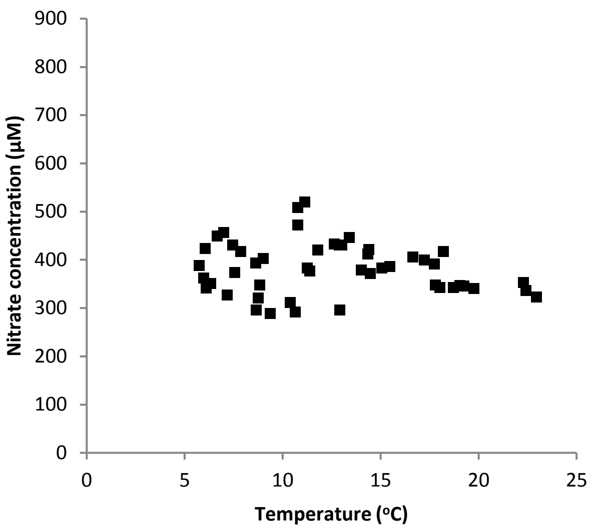


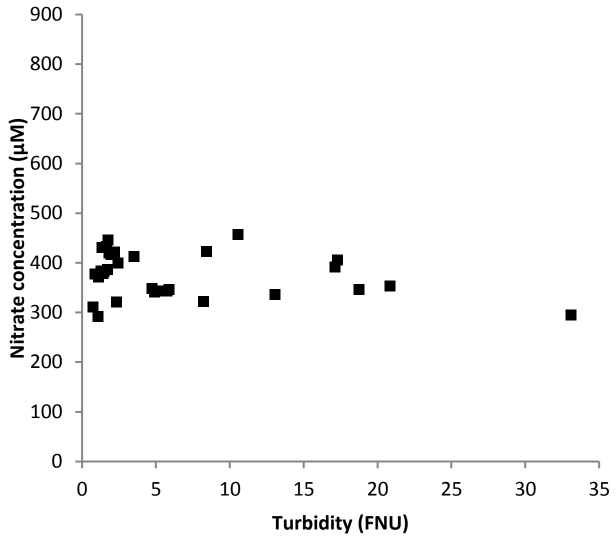


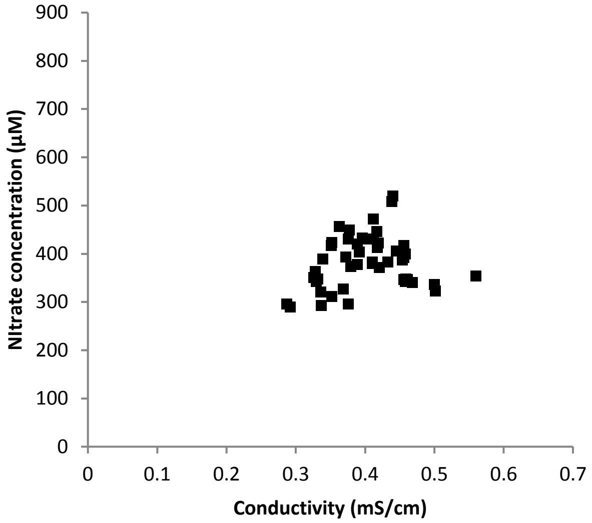
Appendix 2 – Relationships between inorganic nutrients and environmental parameters

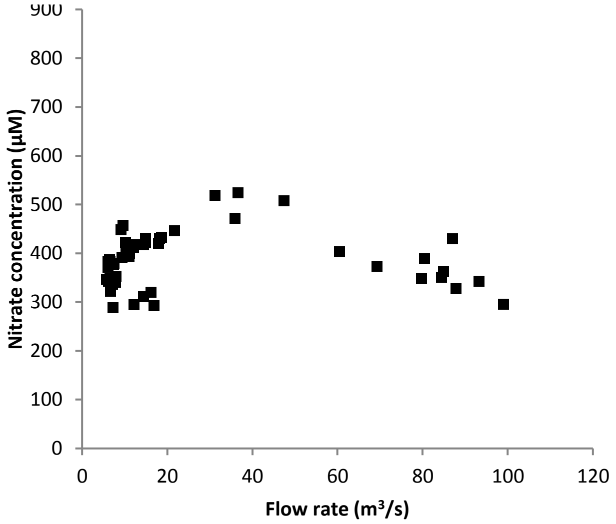
2:1 Knapp Mill

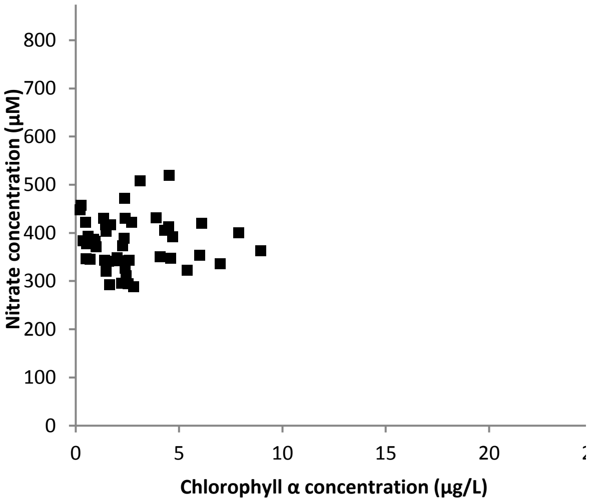
2:1:1 Nitrate

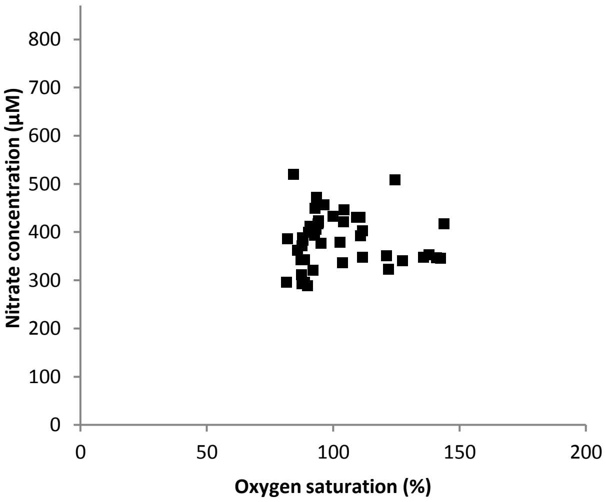




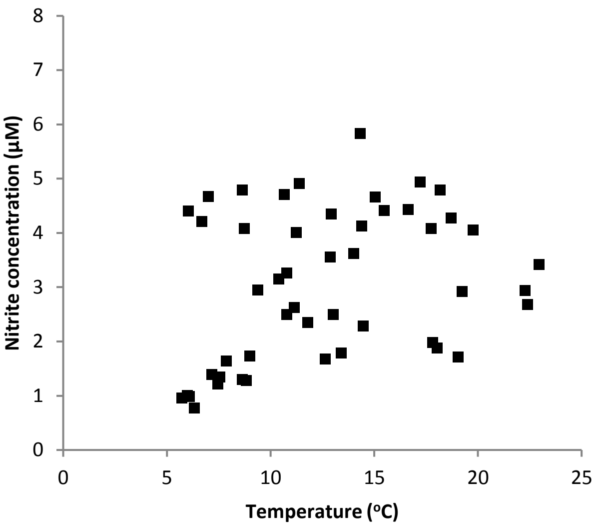


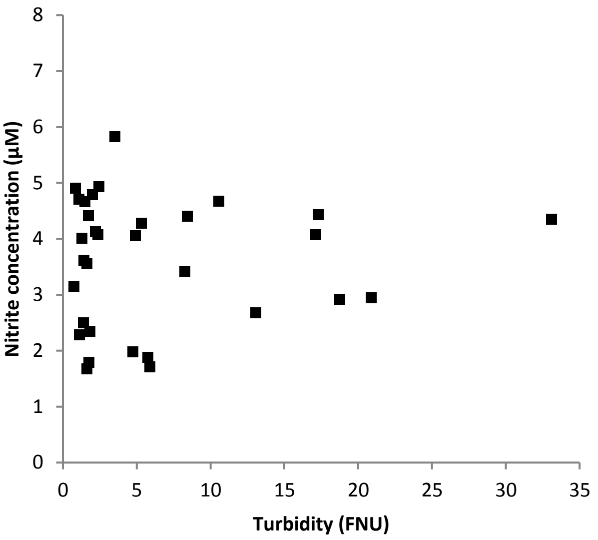


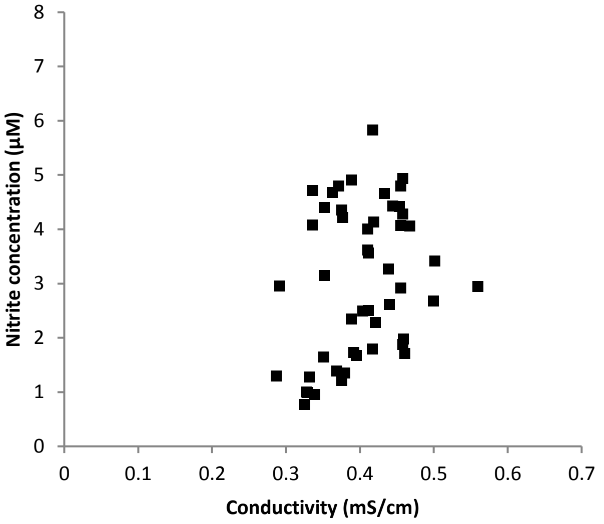


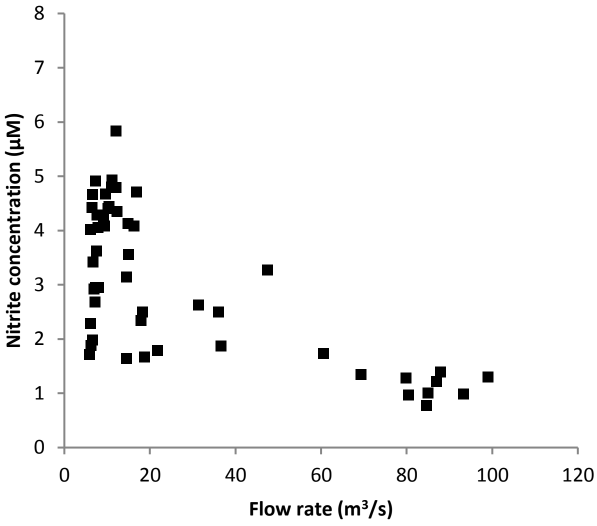


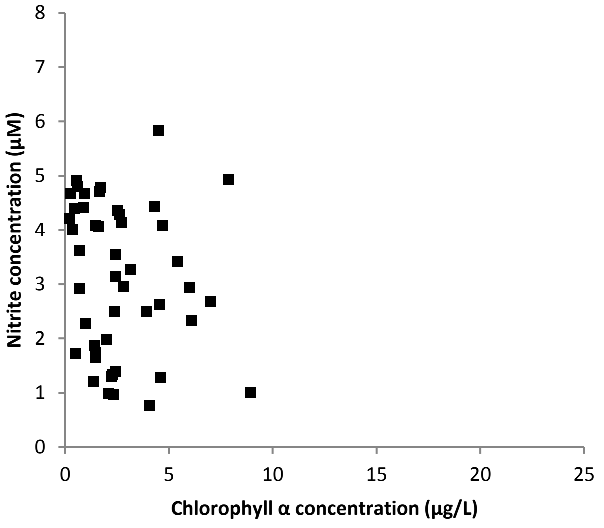
2:1:2 Nitrite

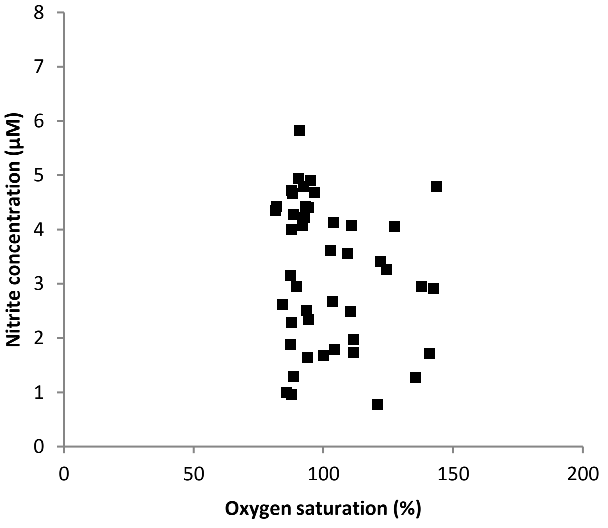

Nitrite concentration (µM)
0
1
2
3
4
5
6
7
8
0
5
10
15
20
25
Temperature (°C)


Nitrite concentration (µM)
0
1
2
3
4
5
6
7
8
0
5
10
15
20
25
30
35
Turbidity (FNU)

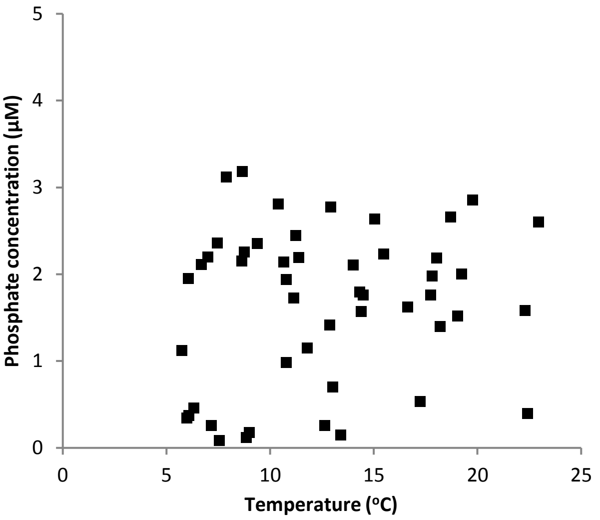

Nitrite concentration (µM)
0
1
2
3
4
5
6
7
8
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Conductivity (mS/cm)

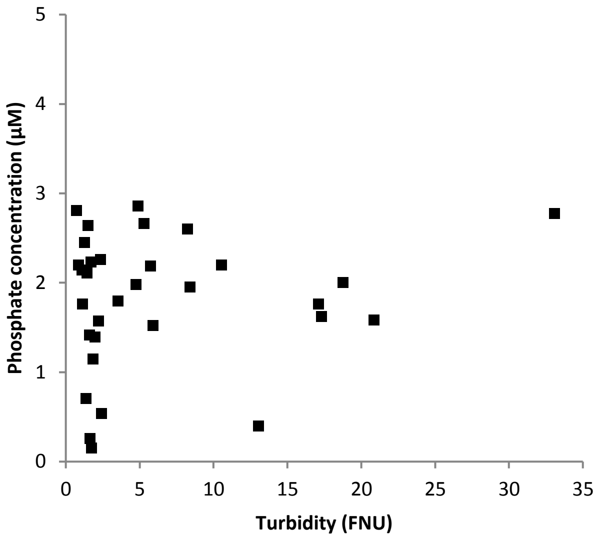

Nitrite concentration (µM)
0
1
2
3
4
5
6
7
8
0
20
40
60
80
100
120
Flow rate (m³/s)

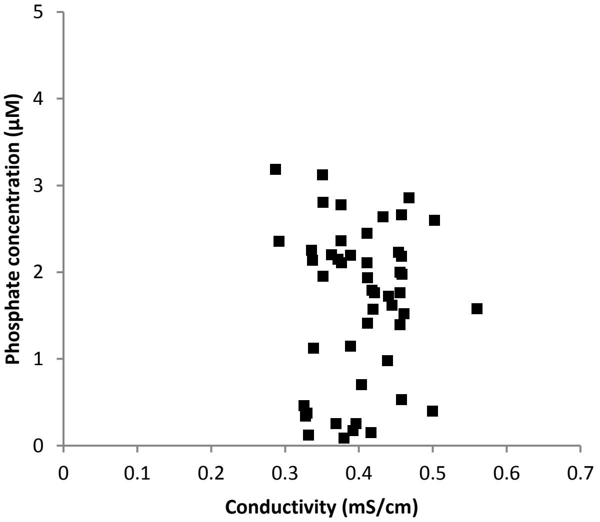

Nitrite concentration (µM)
0
1
2
3
4
5
6
7
8
0
5
10
15
20
25
Chlorophyll α concentration (µg/L)

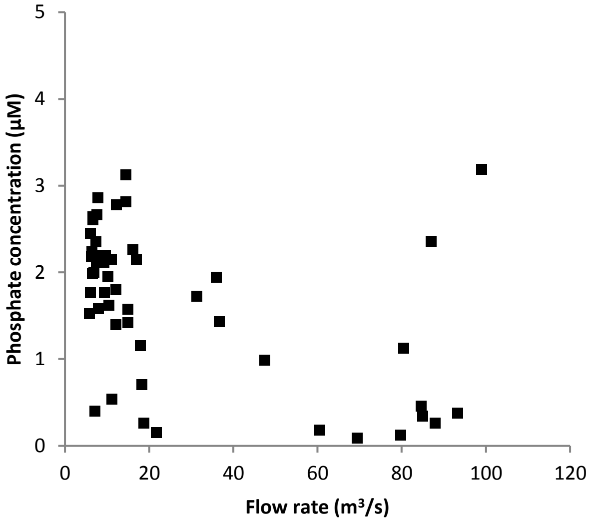

Nitrite concentration (µM)
0
1
2
3
4
5
6
7
8
0
50
100
150
200
Oxygen saturation (%)

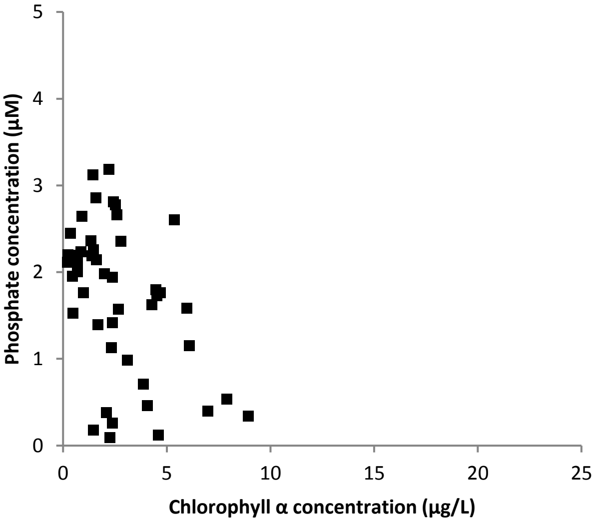
2:1:3 Soluble reactive phosphorus (SRP)

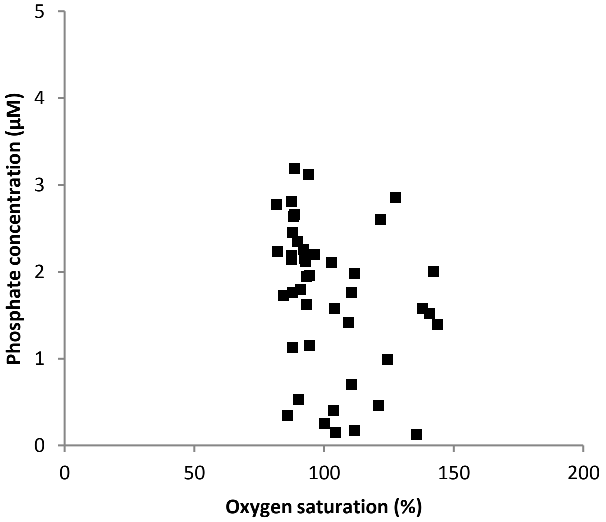




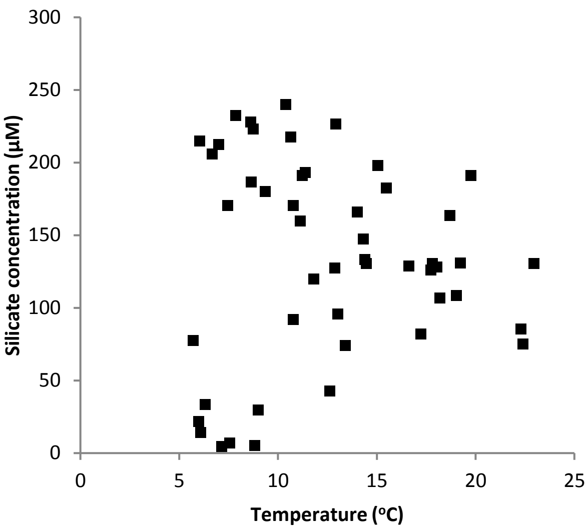


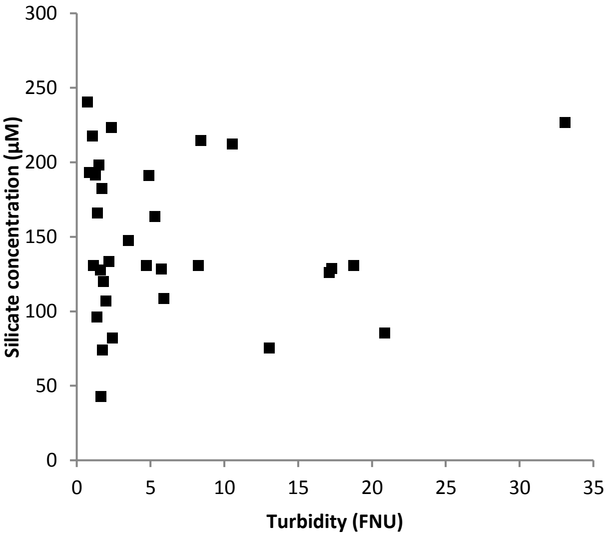


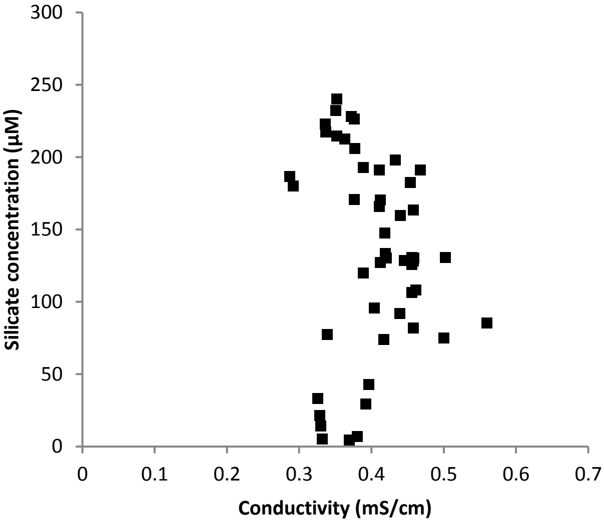


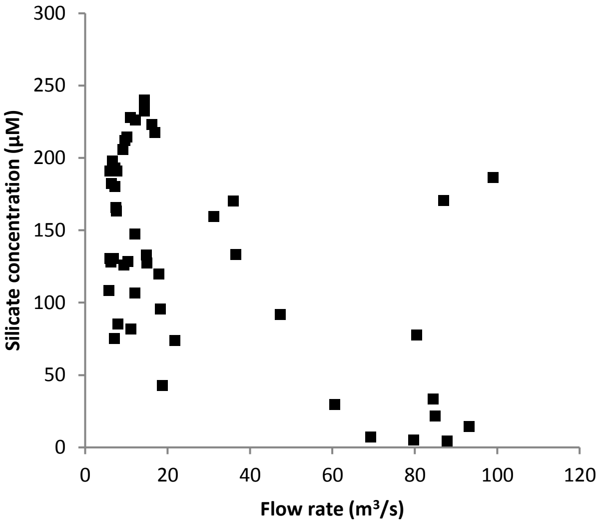


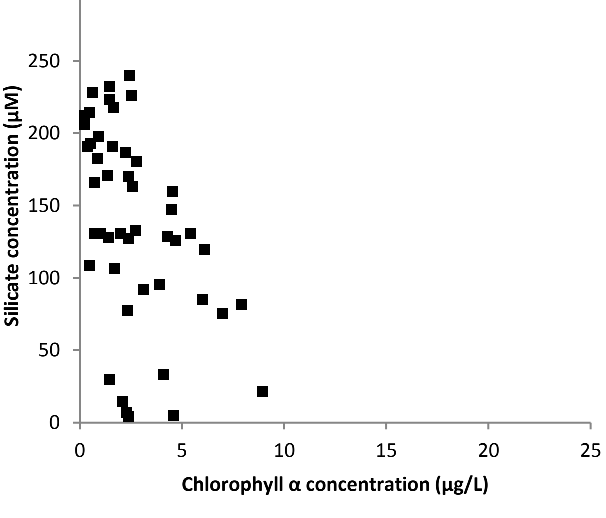
2:1:4 Silicate

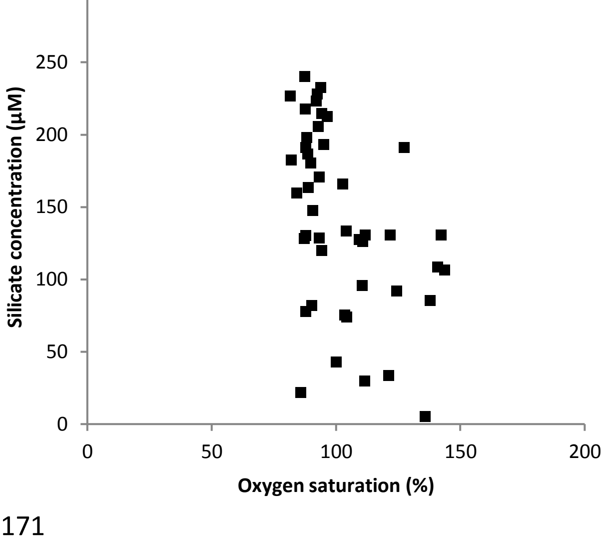






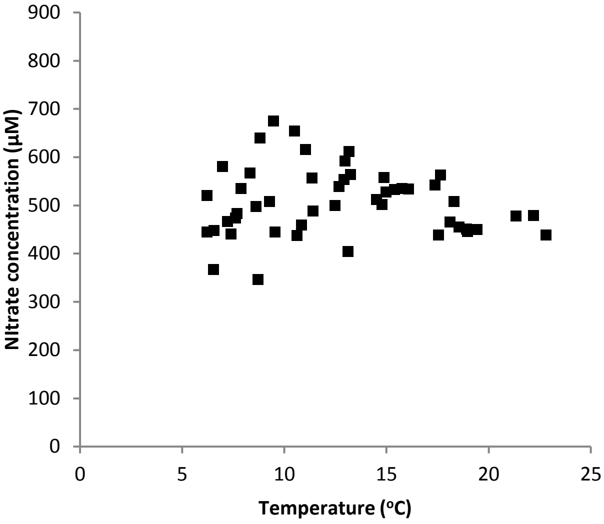


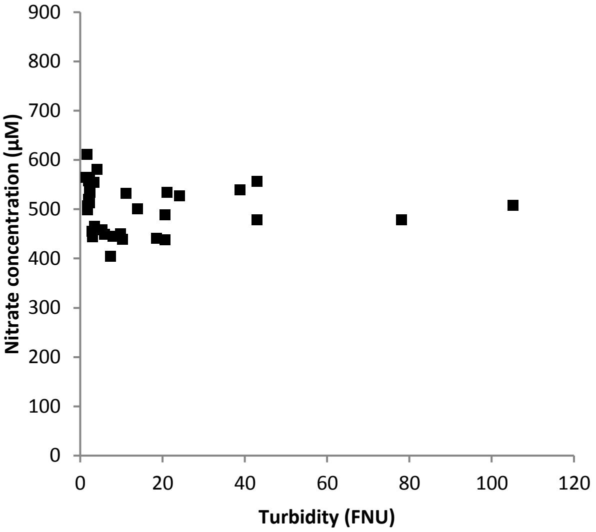


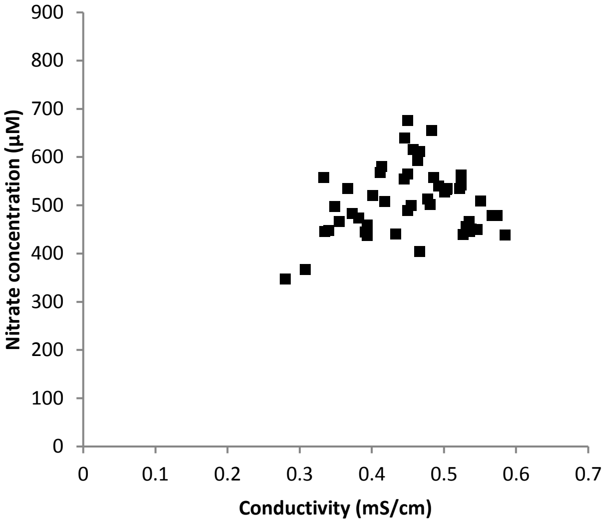


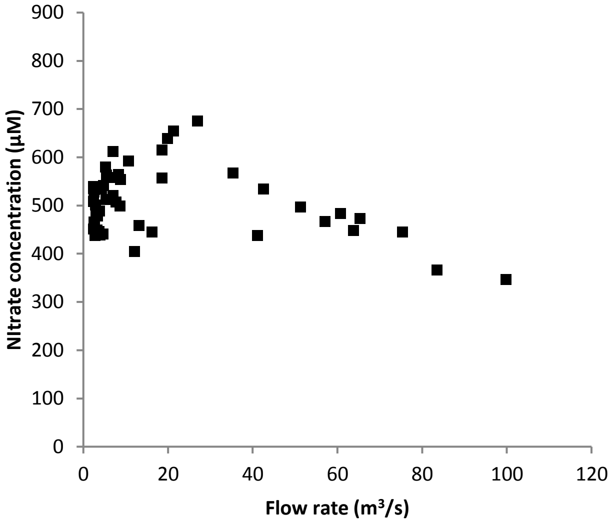
2:2 Throop

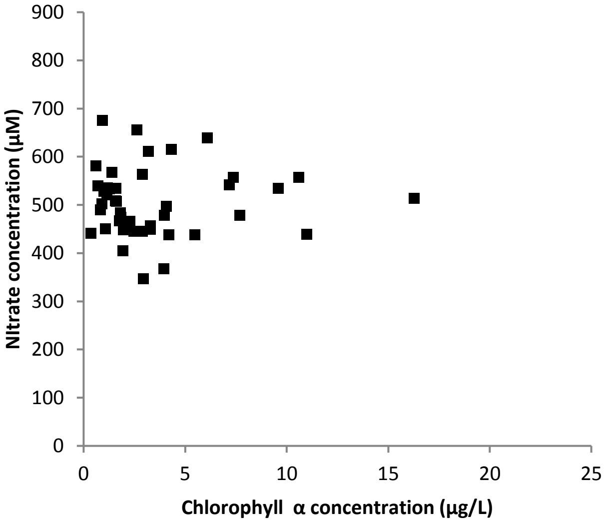
2:2:1 Nitrate

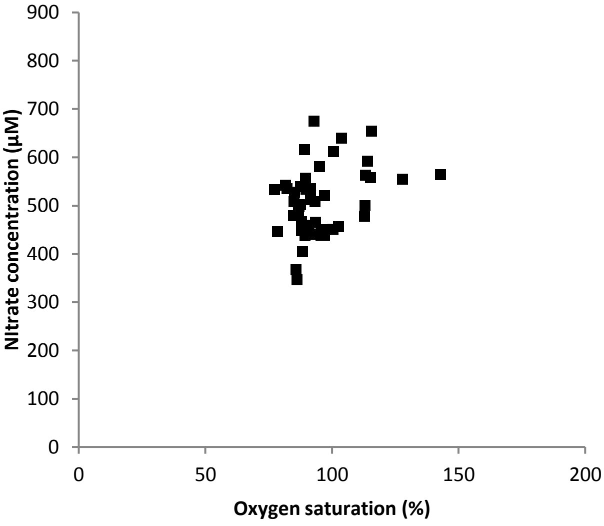




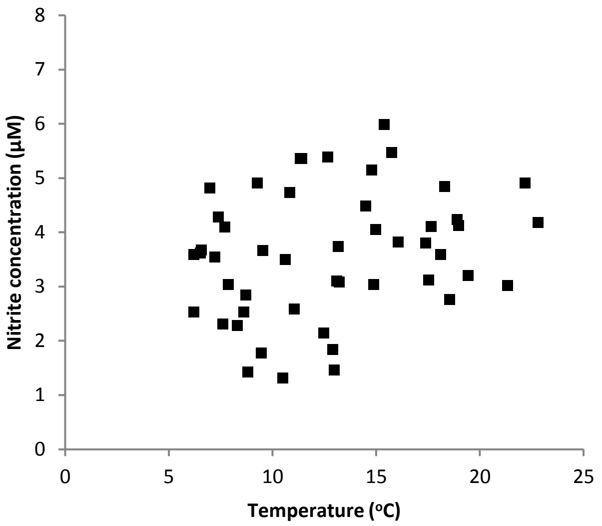


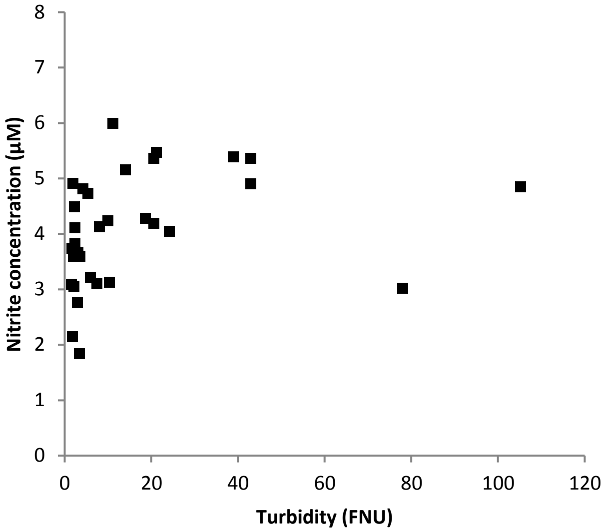


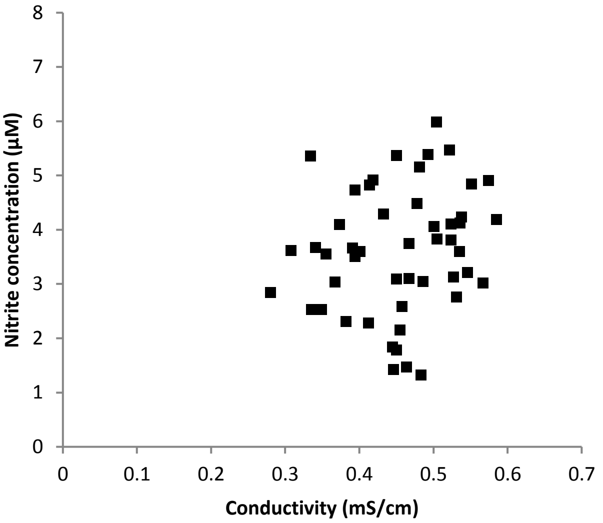


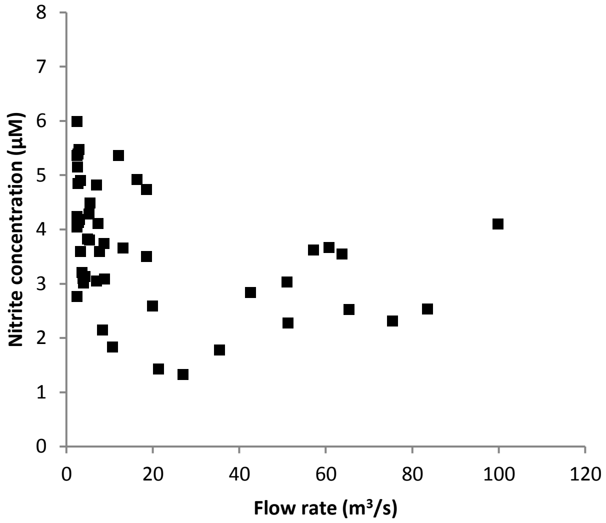


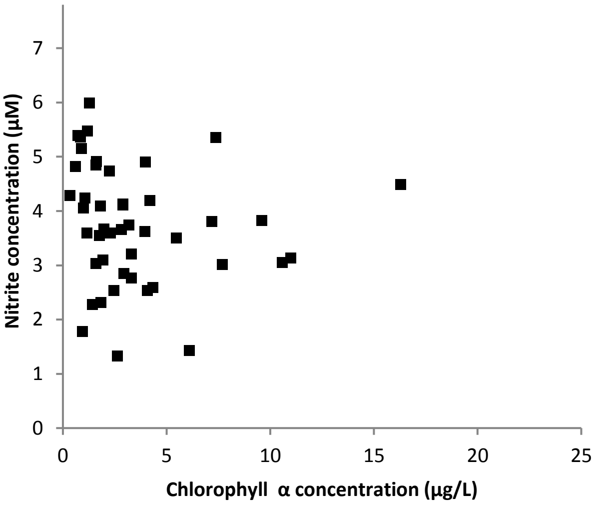
2:2:2 Nitrite

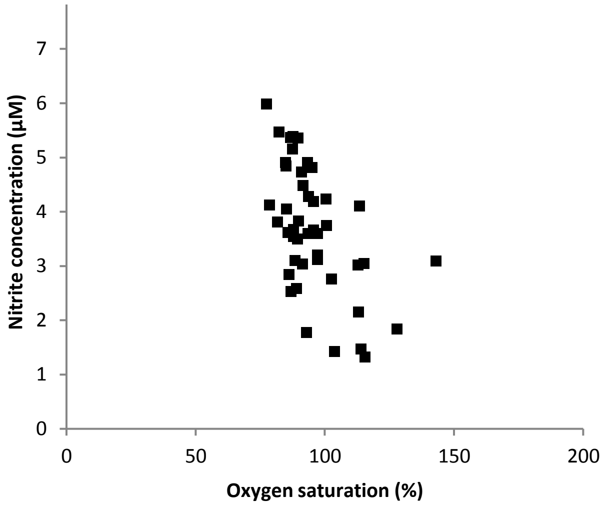

Nitrite concentration (µM)
0
1
2
3
4
5
6
7
8
0
5
10
15
20
25
Temperature (°C)


Nitrite concentration (µM)
0
1
2
3
4
5
6
7
8
0
20
40
60
80
100
120
Turbidity (FNU)

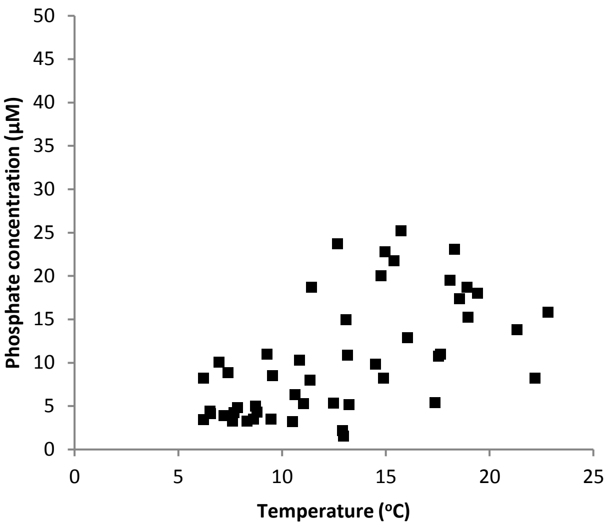

Nitrite concentration (µM)
0
1
2
3
4
5
6
7
8
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Conductivity (mS/cm)

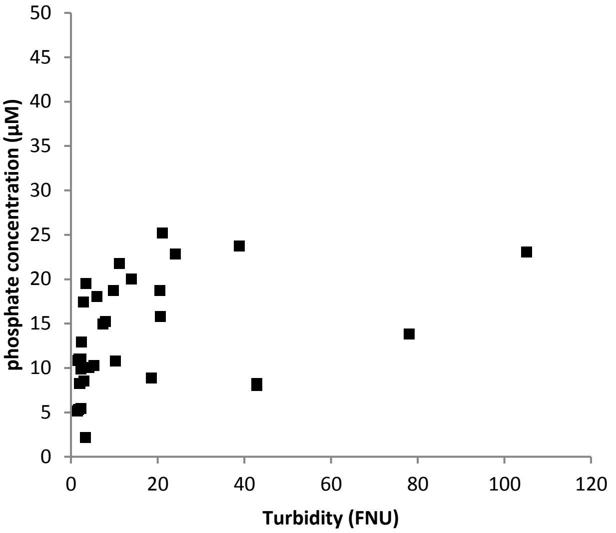

Nitrite concentration (µM)
0
1
2
3
4
5
6
7
8
0
20
40
60
80
100
120
Flow rate (m³/s)

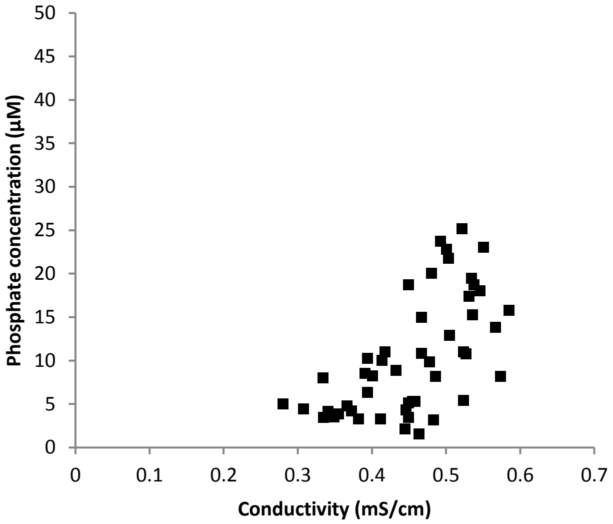

Nitrite concentration (µM)
0
1
2
3
4
5
6
7
0
5
10
15
20
25
Chlorophyll α concentration (µg/L)

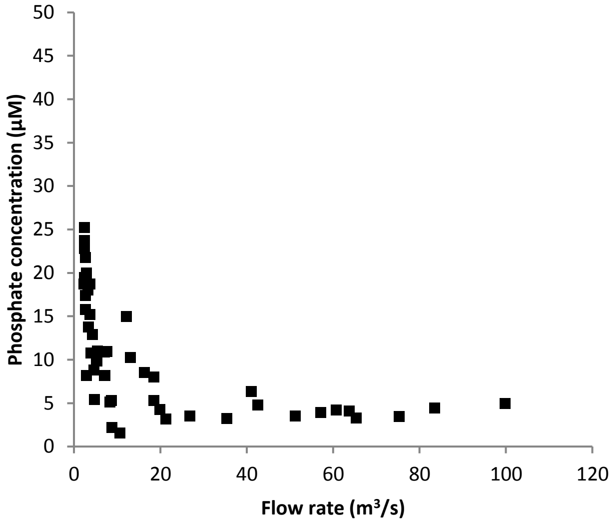

Nitrite concentration (µM)
0
1
2
3
4
5
6
7
0
50
100
150
200
Oxygen saturation (%)

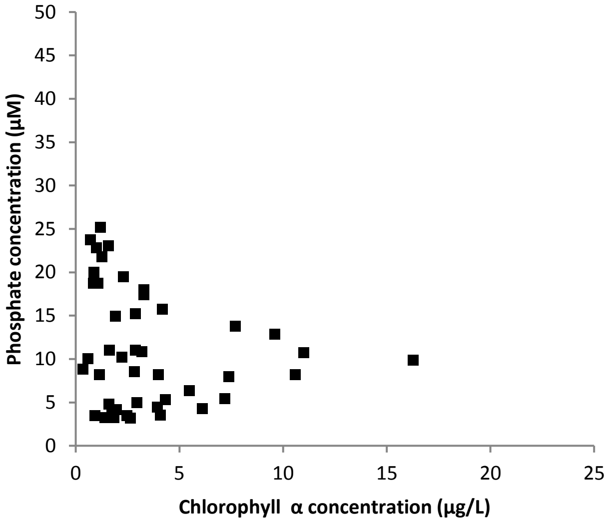
2:2:3 Soluble reactive phosphorus (SRP)

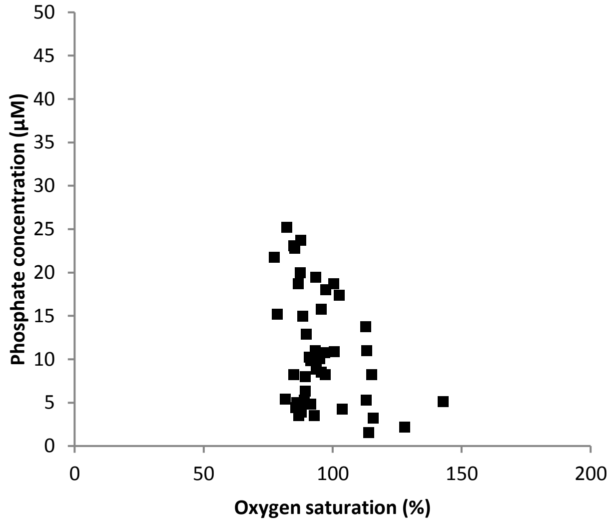




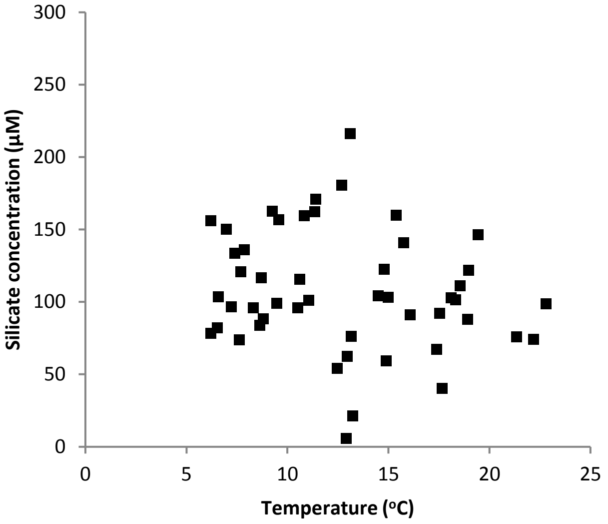


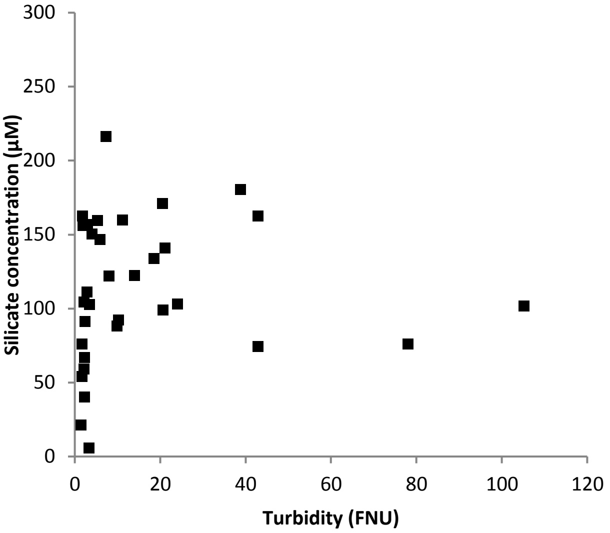


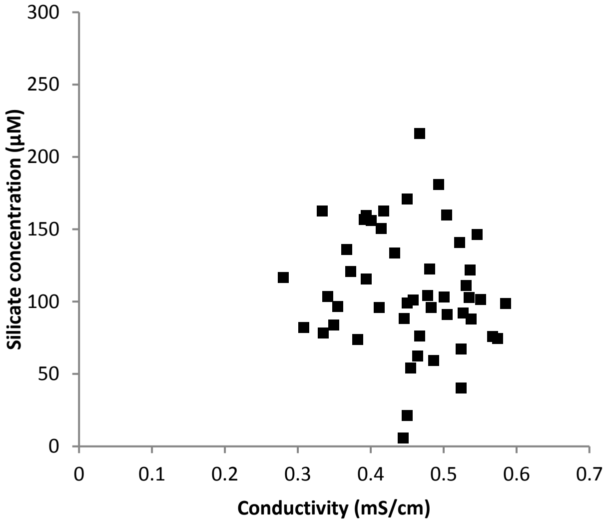


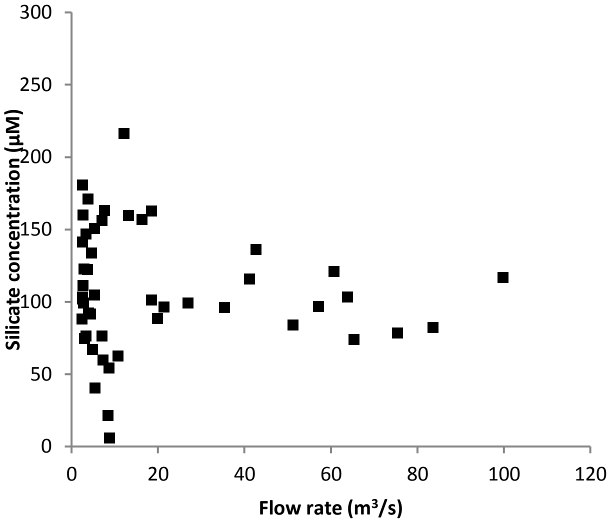


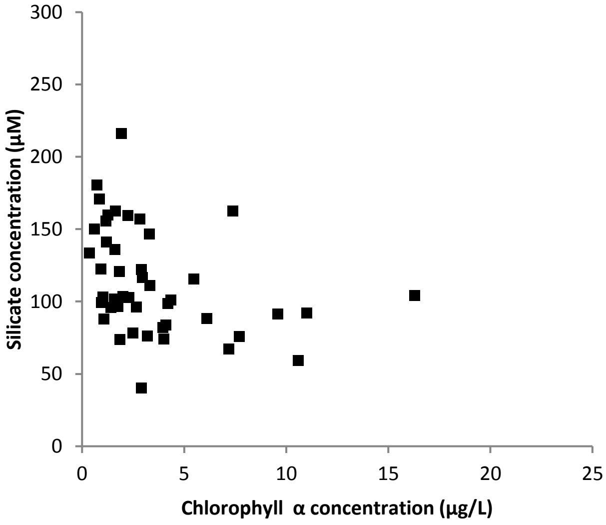
2:2:4 Silicate

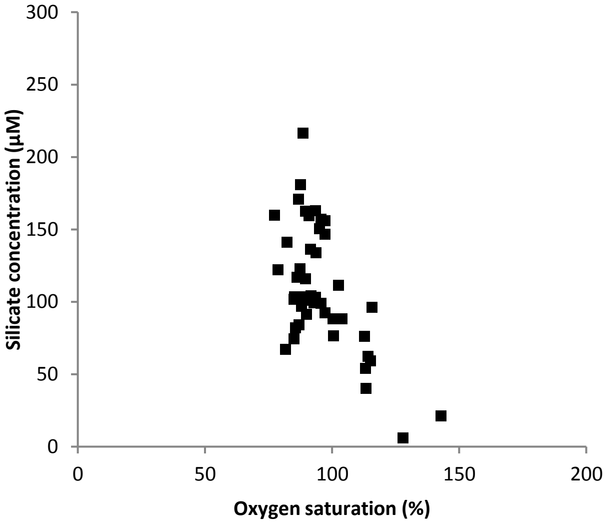






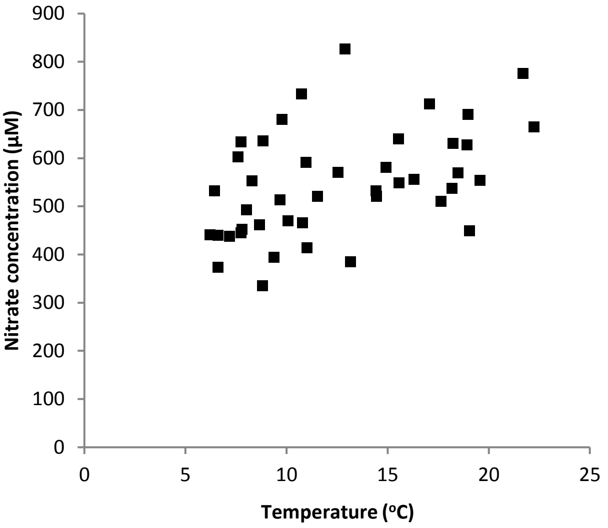


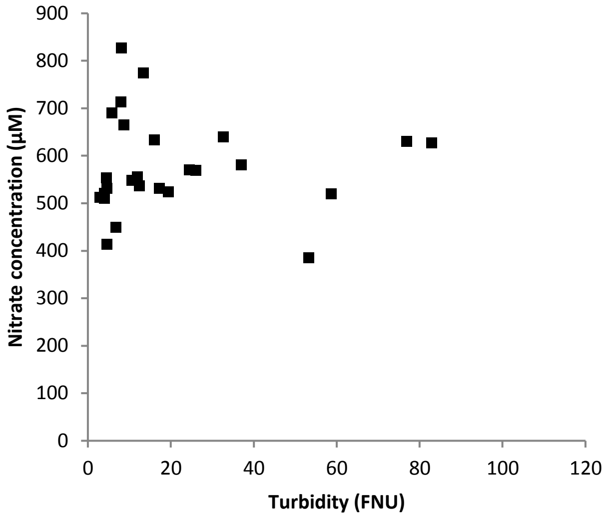


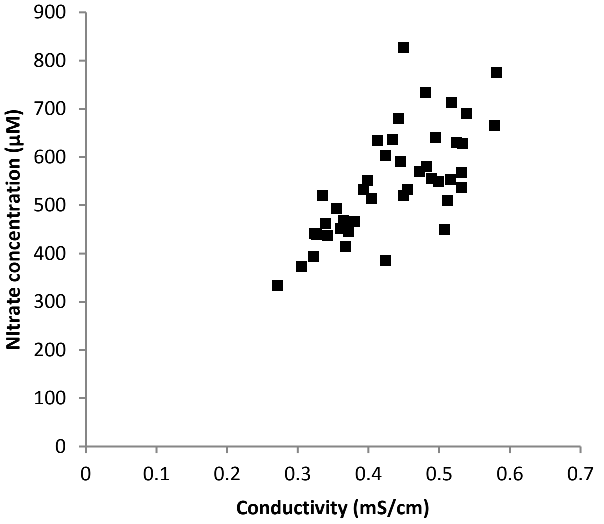


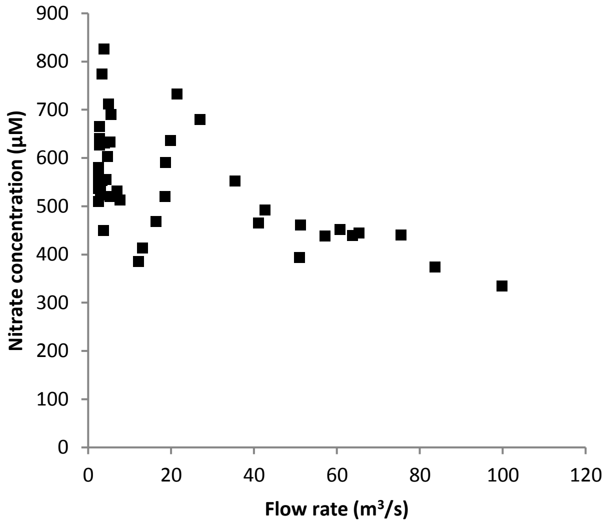
2:3 Iford

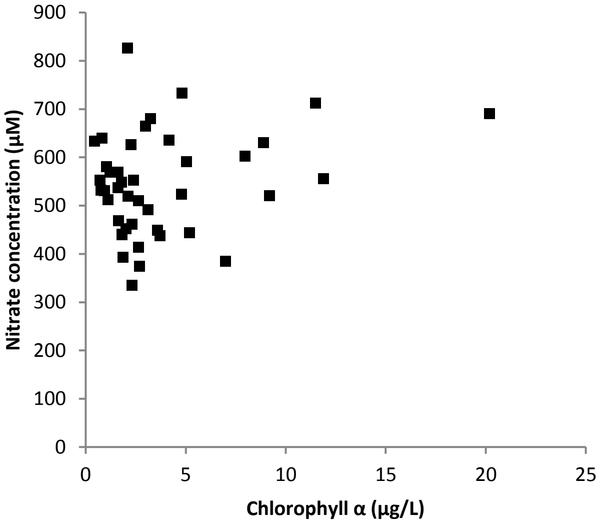
2:3:1 Nitrate

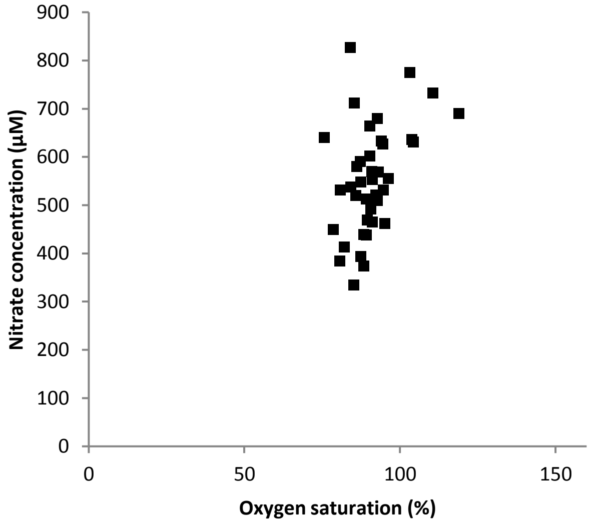




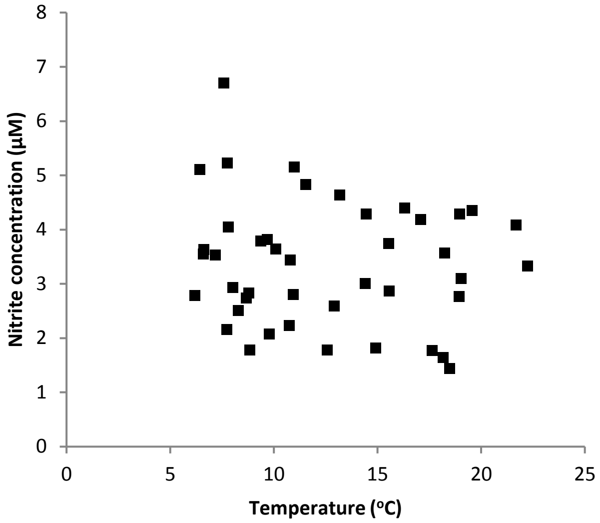


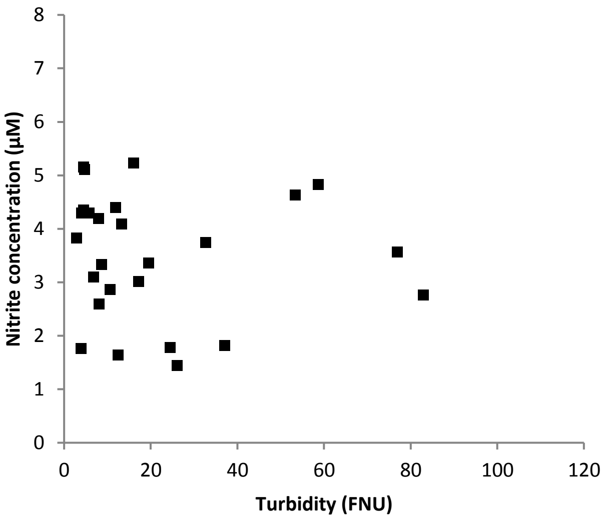


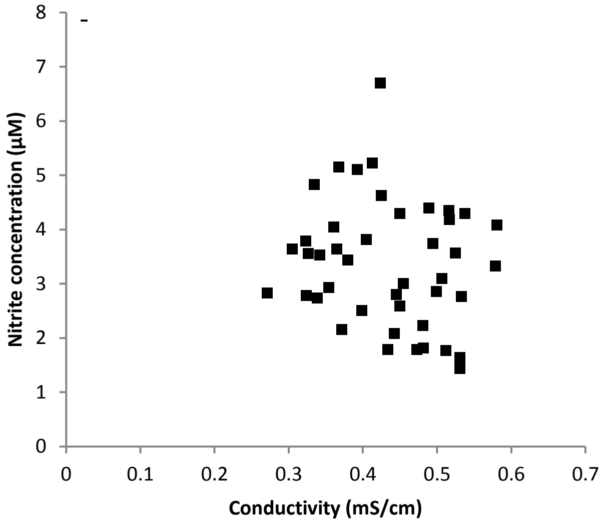


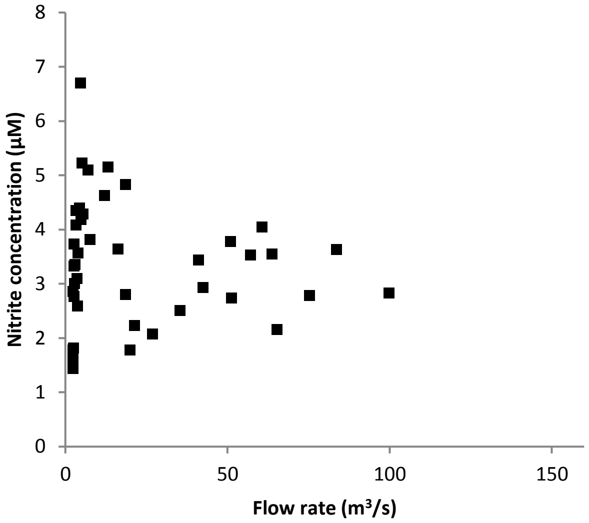


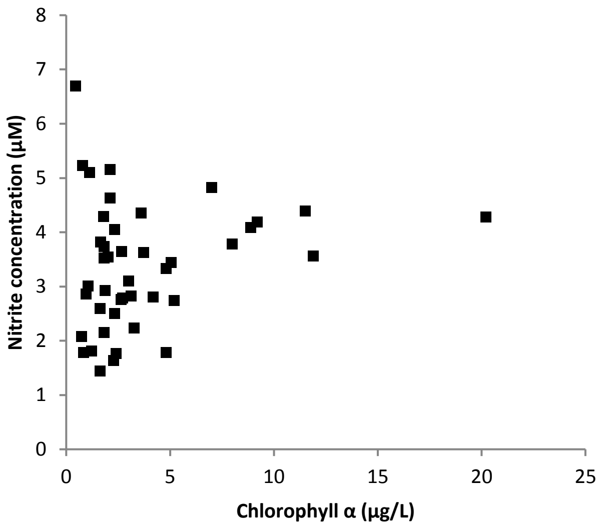
2:3:2 Nitrite

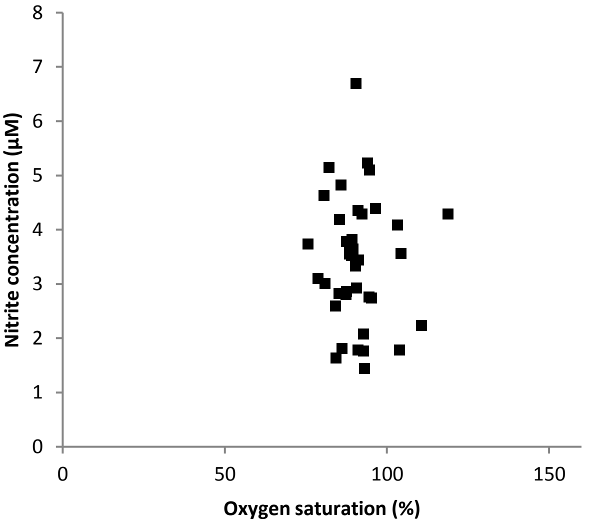

Nitrite concentration (µM)
0
1
2
3
4
5
6
7
8
0
5
10
15
20
25
Temperature (°C)


Nitrite concentration (µM)
0
1
2
3
4
5
6
7
8
0
20
40
60
80
100
120
Turbidity (FNU)

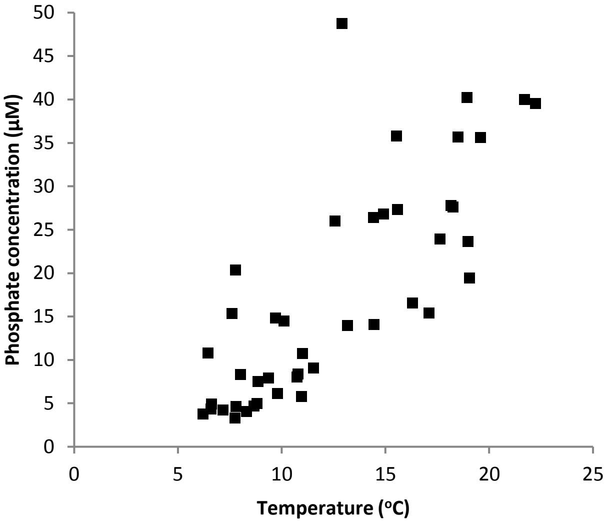

Nitrite concentration (µM)
0
1
2
3
4
5
6
7
8
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Conductivity (mS/cm)

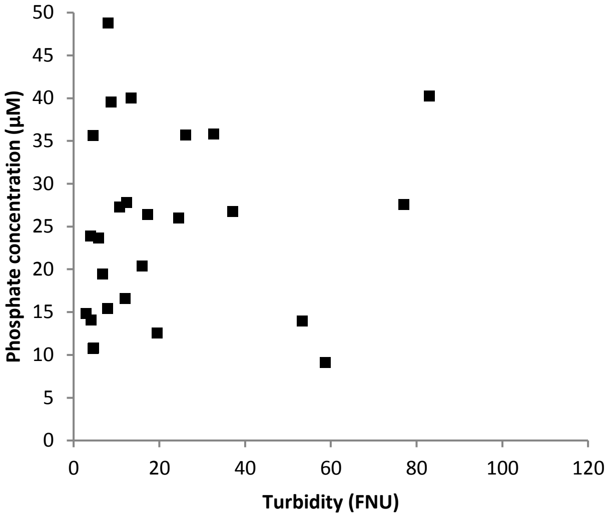

Nitrite concentration (µM)
0
1
2
3
4
5
6
7
8
0
50
100
150
Flow rate (m³/s)

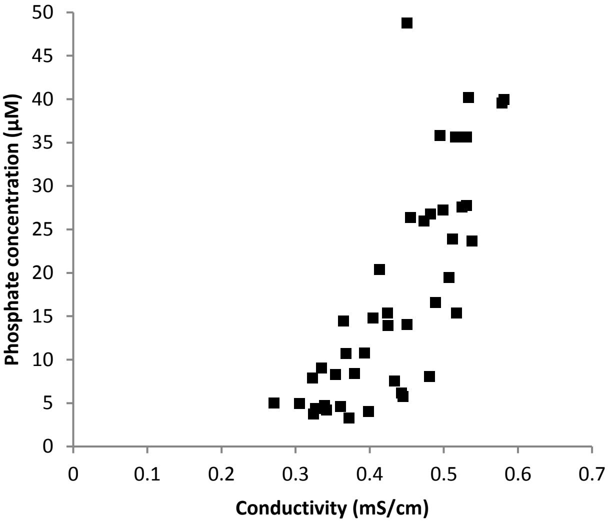

Nitrite concentration (µM)
0
1
2
3
4
5
6
7
8
0
5
10
15
20
25
Chlorophyll α (µg/L)

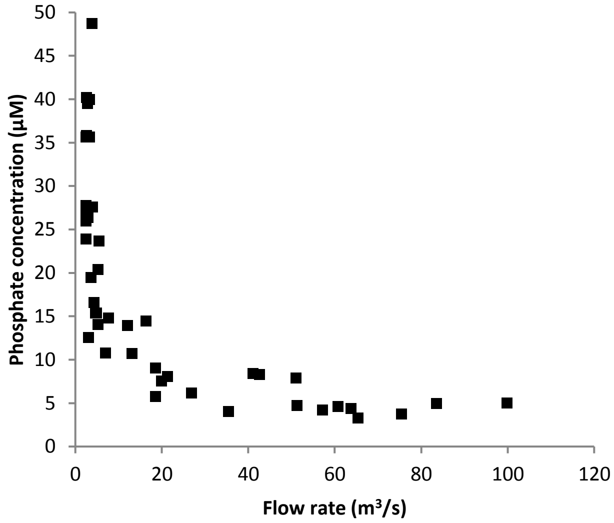

Nitrite concentration (µM)
0
1
2
3
4
5
6
7
8
0
50
100
150
Oxygen saturation (%)

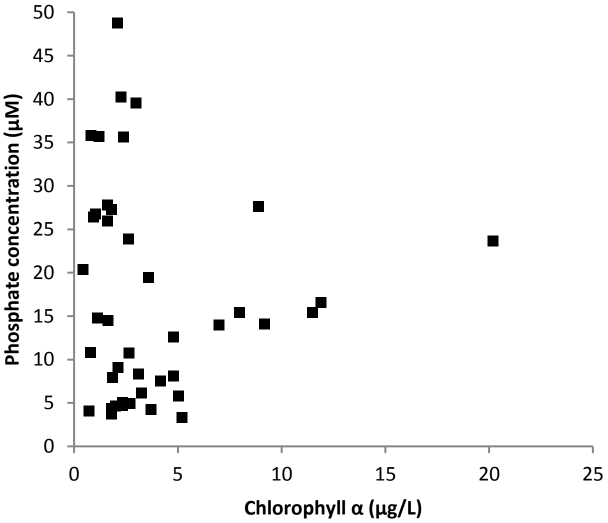
2:3:3 Soluble reactive phosphorus (SRP)

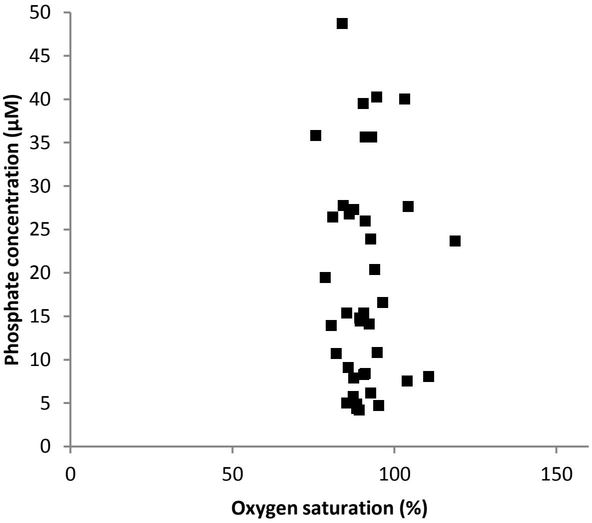




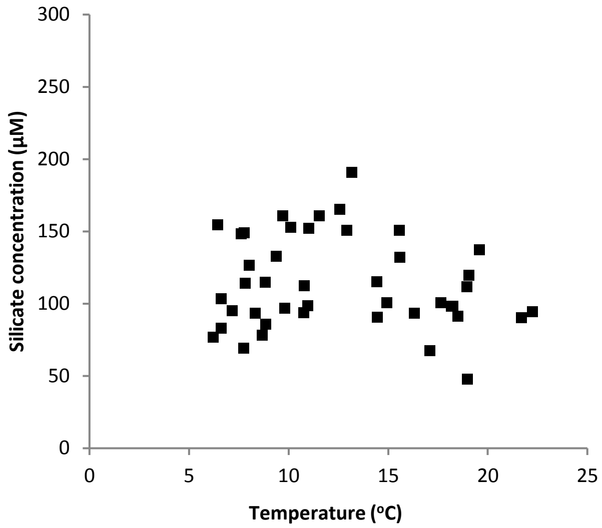


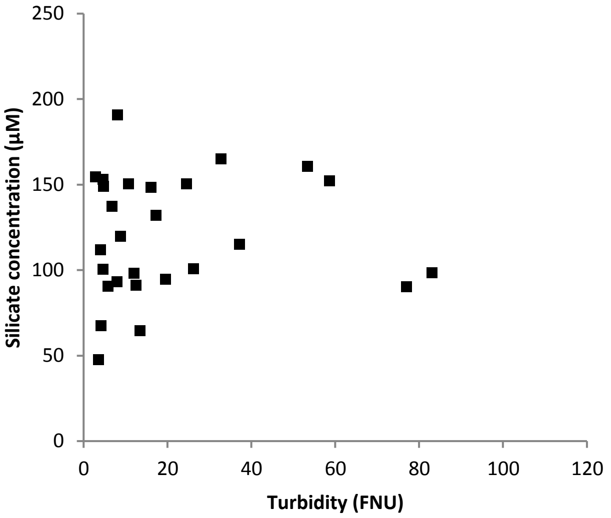


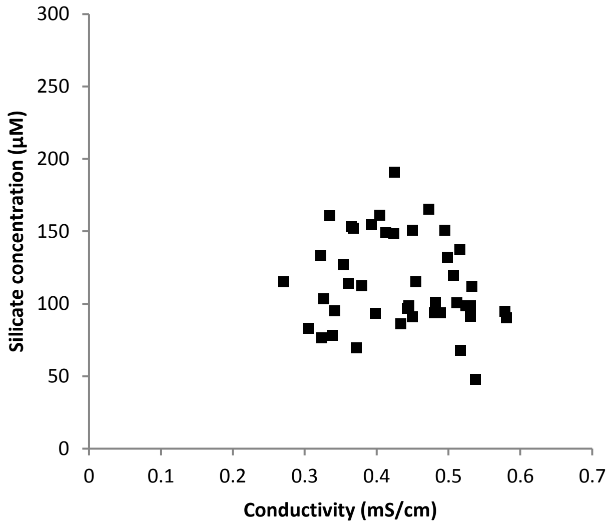


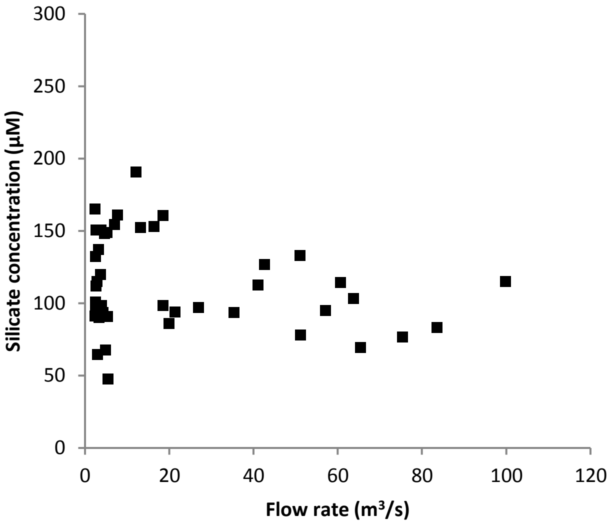


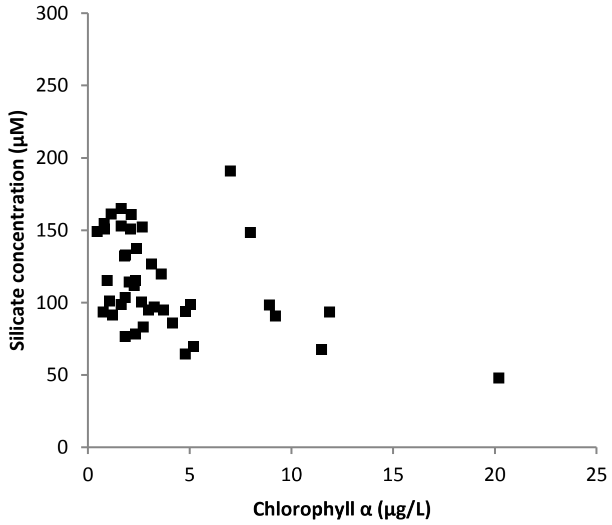
2:3:4 Silicate

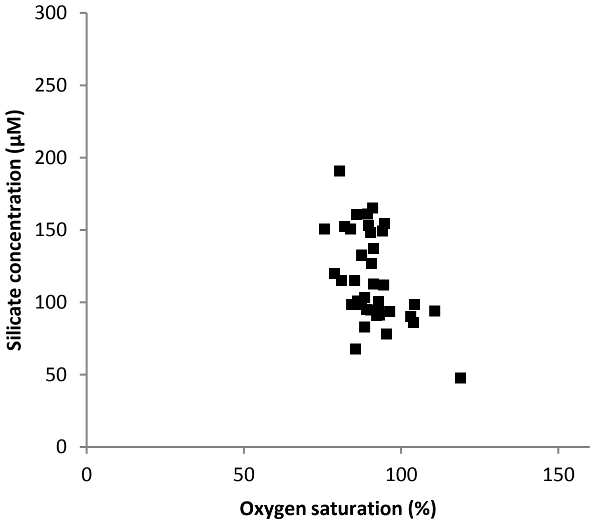






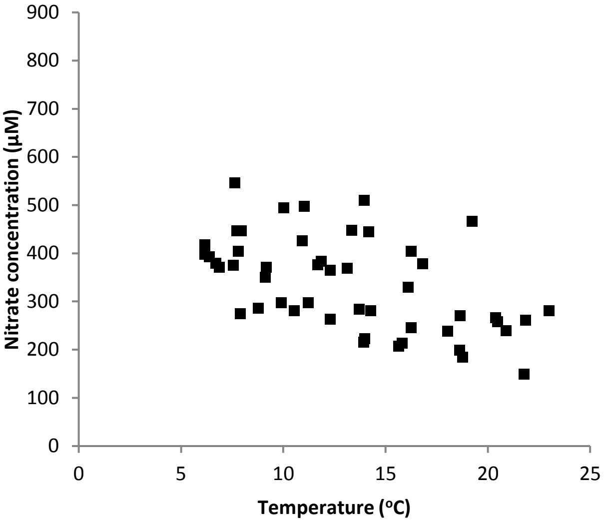


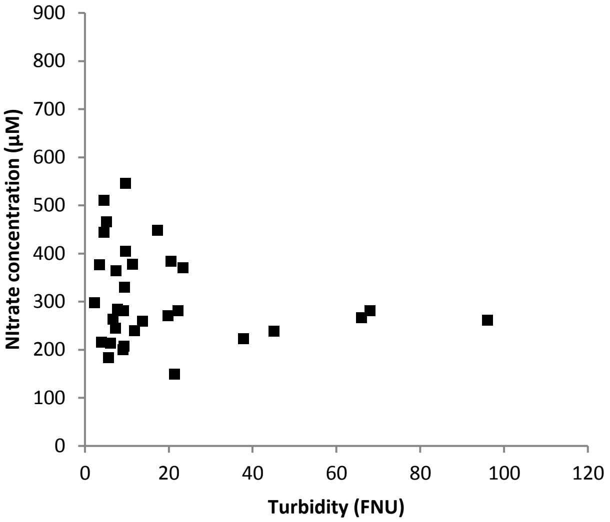


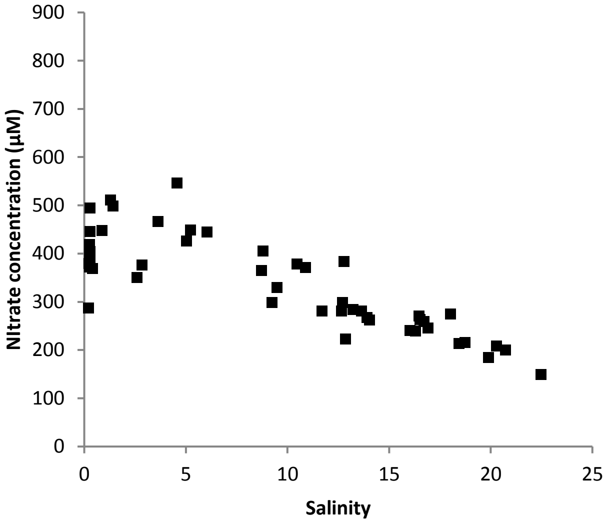


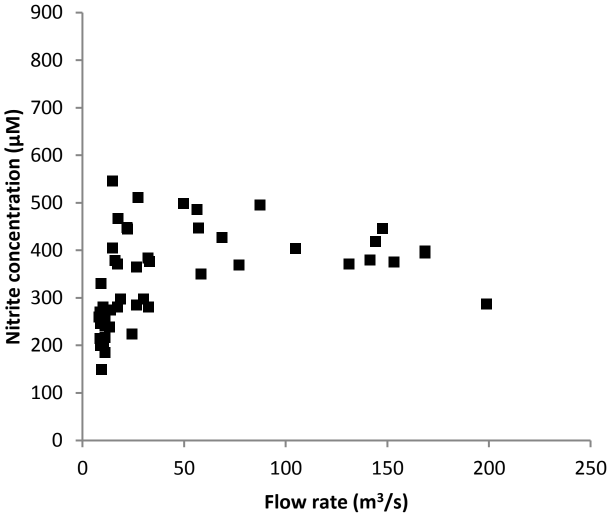
2:4 Mudeford Quay

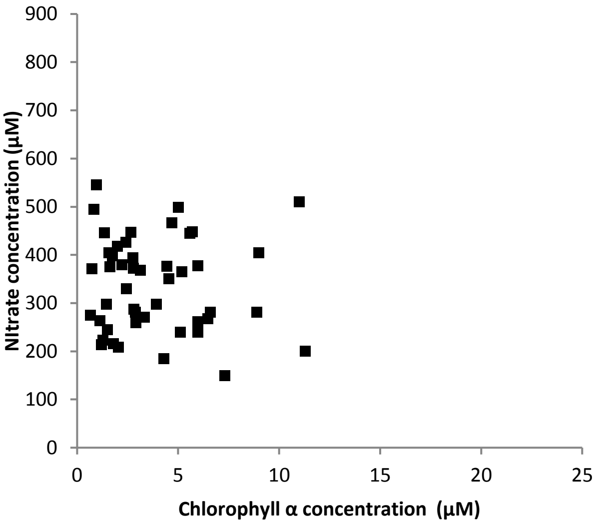
2:4:1 Nitrate

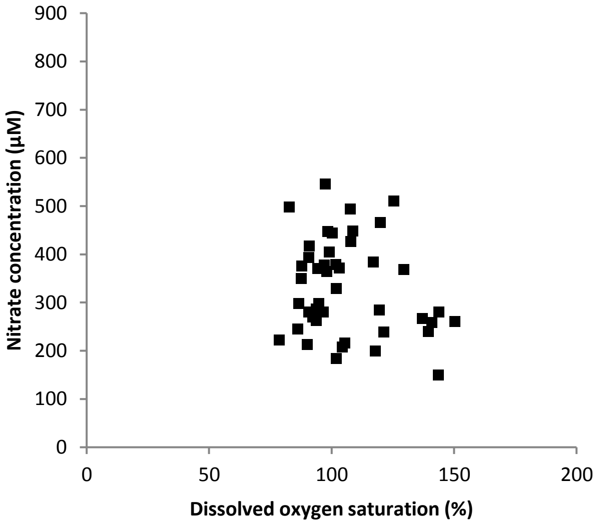




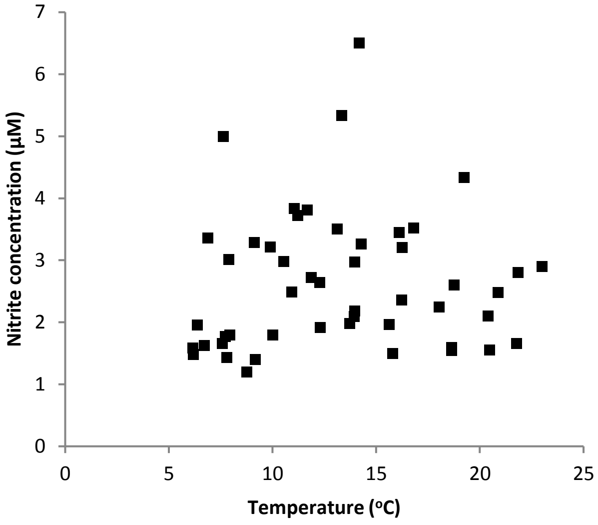


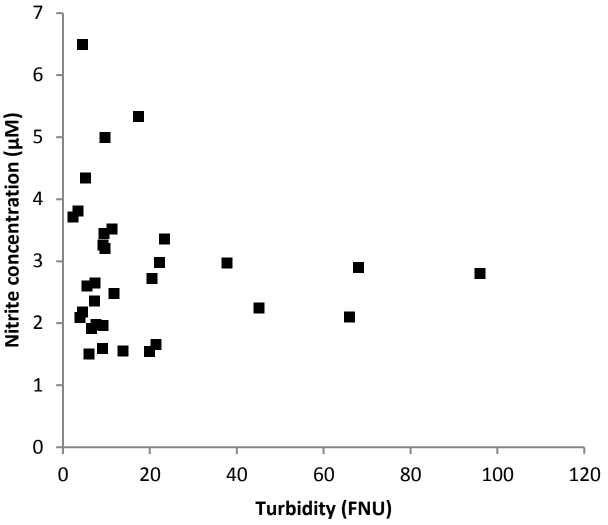


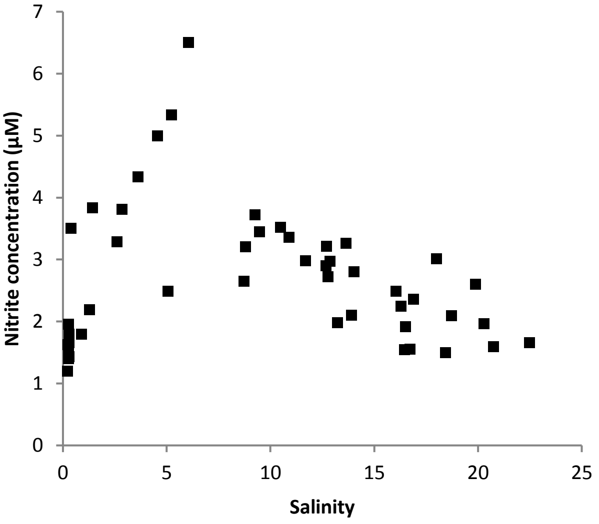


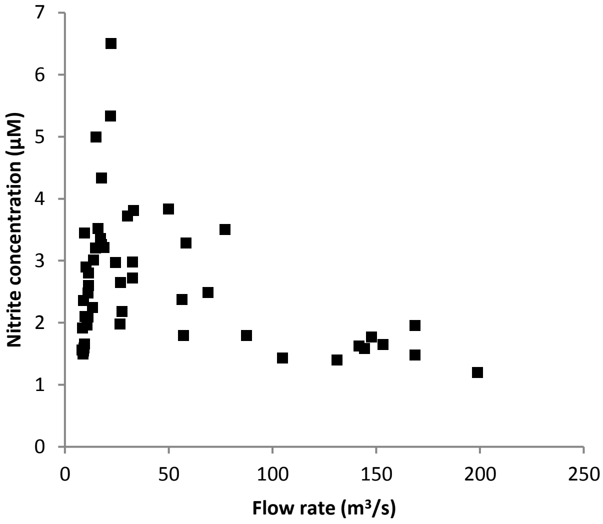


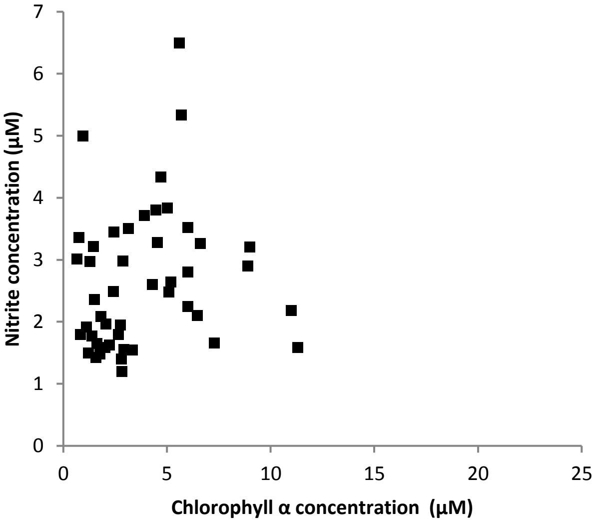
2:4:2 Nitrite

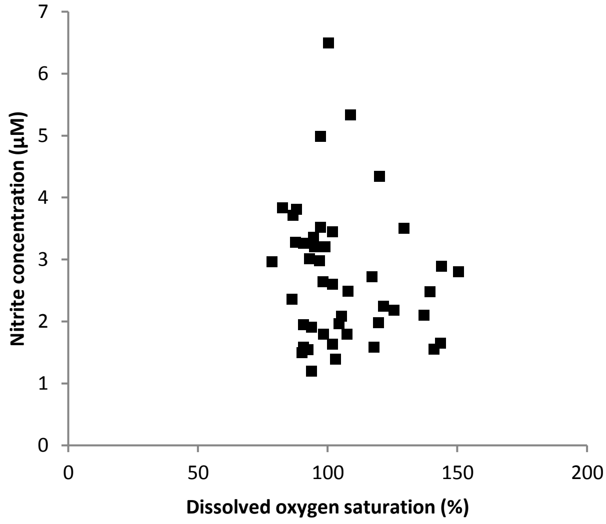

Nitrite concentration (µM)
Temperature (°C)
0
1
2
3
4
5
6
7
0
5
10
15
20
25


Nitrite concentration (µM)
Turbidity (FNU)
0
1
2
3
4
5
6
7
0
20
40
60
80
100
120

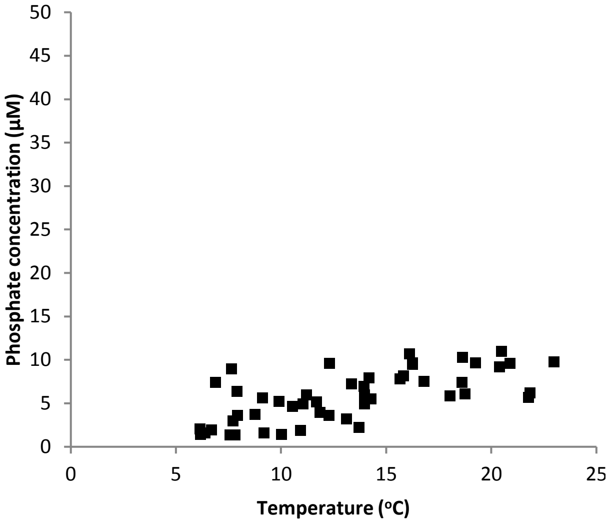

Nitrite concentration (µM)
Salinity
0
1
2
3
4
5
6
7
0
5
10
15
20
25

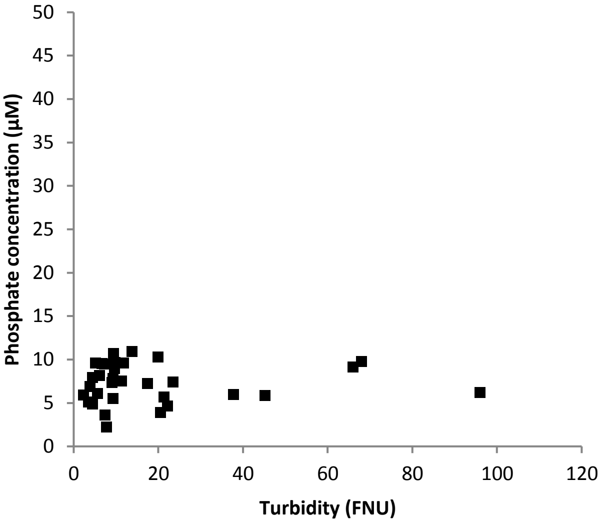

Nitrite concentration (µM)
Flow rate (m³/s)
0
1
2
3
4
5
6
7
0
50
100
150
200
250

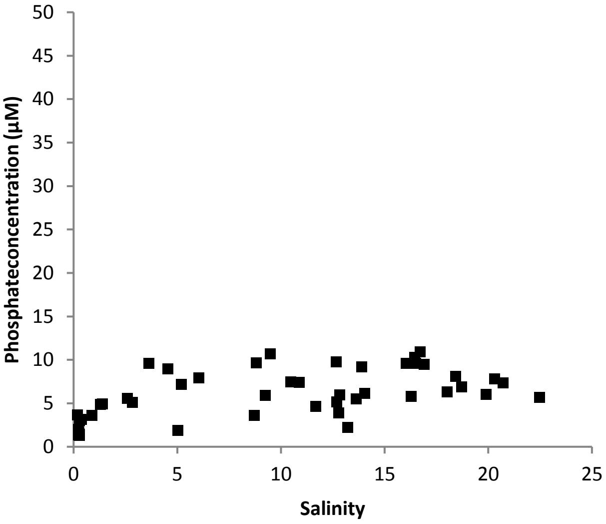

Nitrite concentration (µM)
Chlorophyll α concentration (µM)
0
1
2
3
4
5
6
7
0
5
10
15
20
25

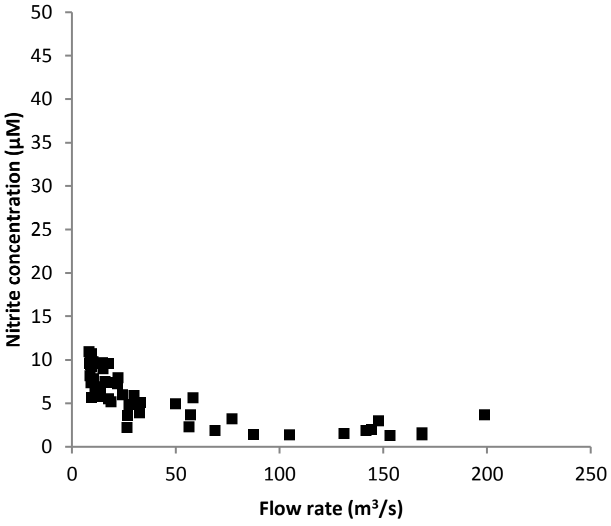

Nitrite concentration (µM)
Dissolved oxygen saturation (%)
0
1
2
3
4
5
6
7
0
50
100
150
200

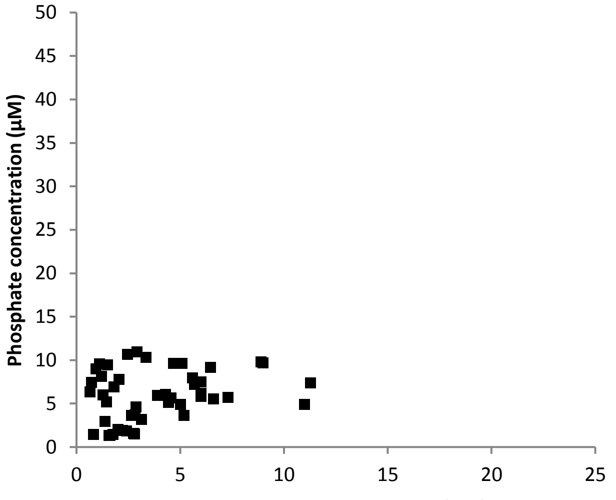
2:4:3 Soluble reactive phosphorus (SRP)

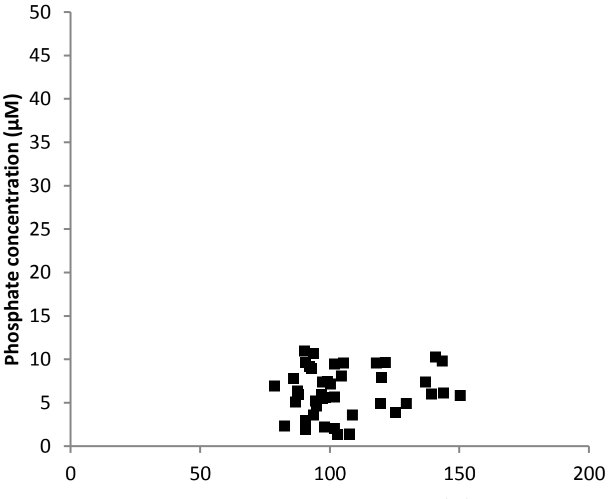




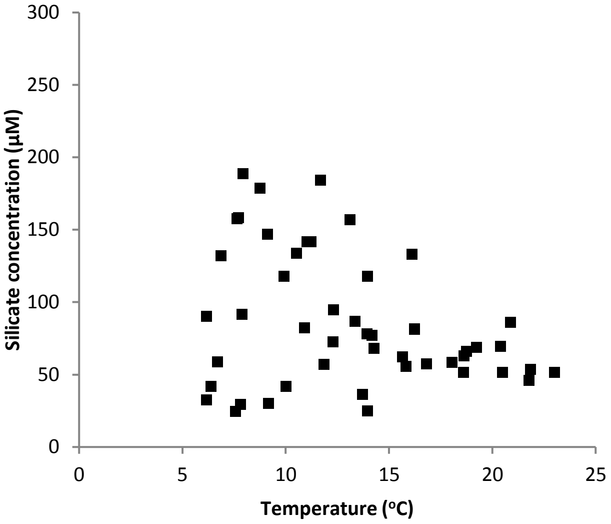


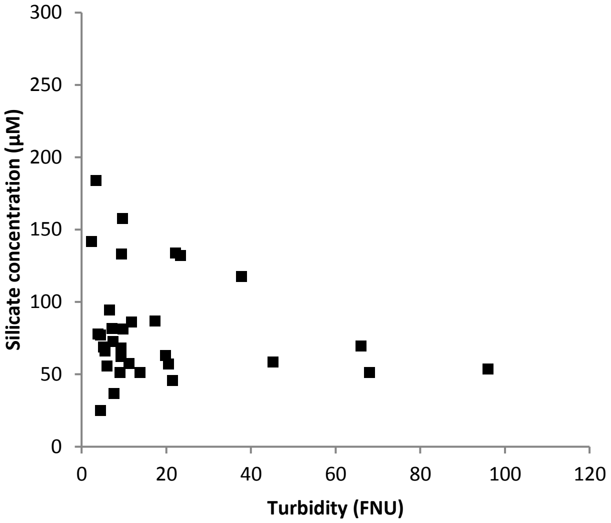


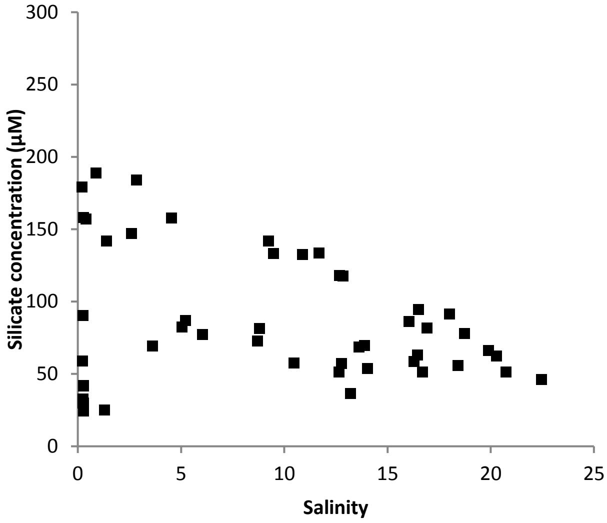


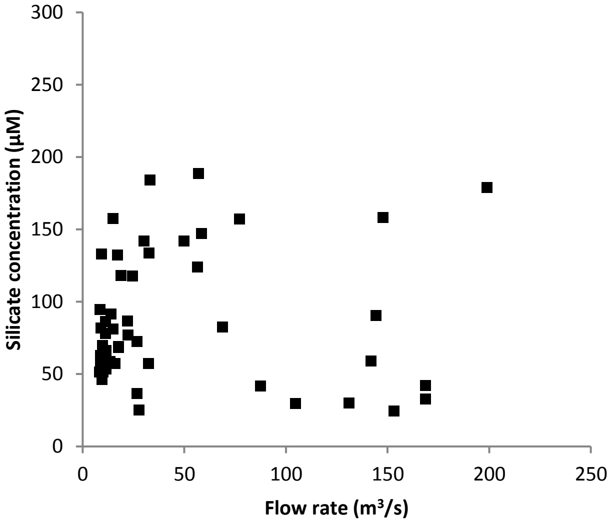


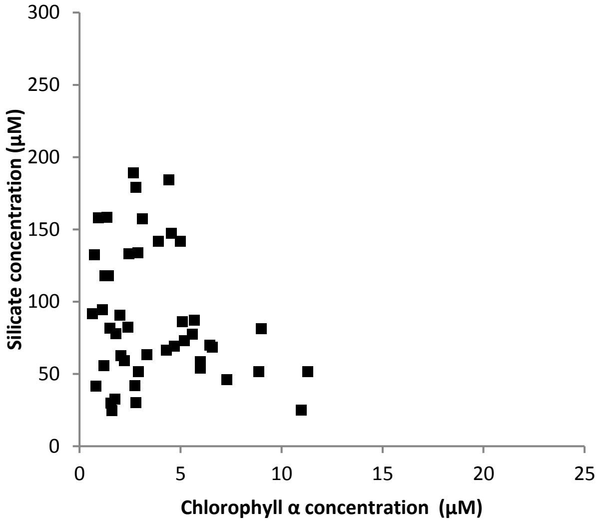
2:4:4 Silicate

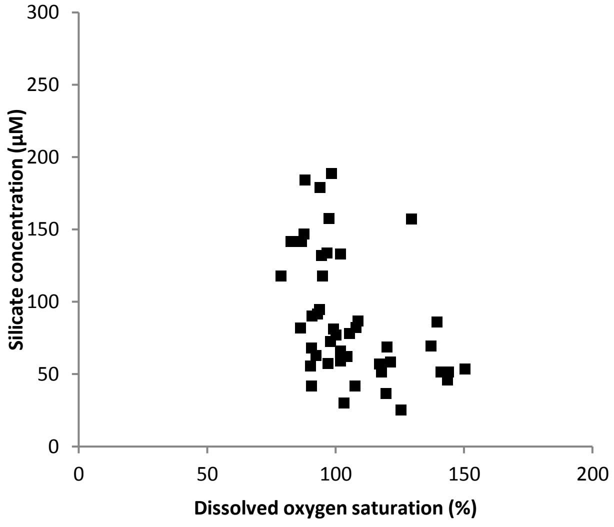






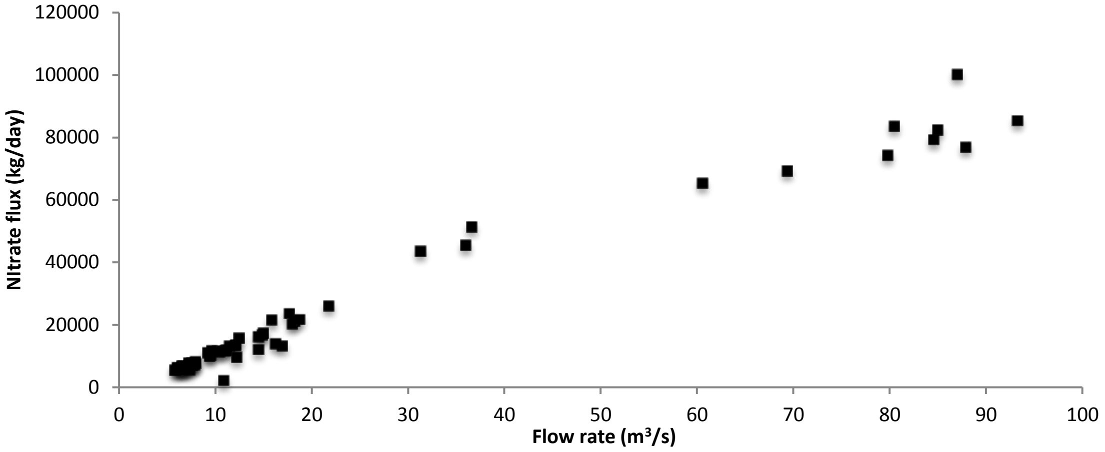


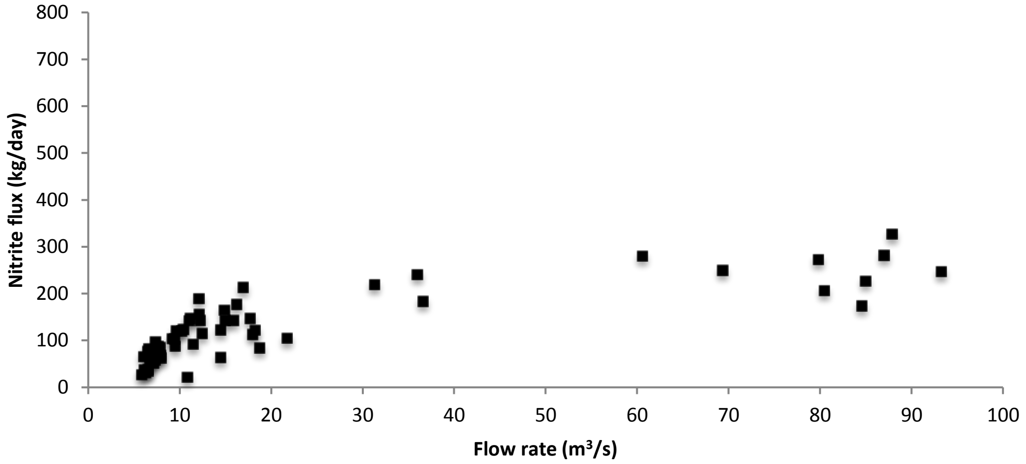


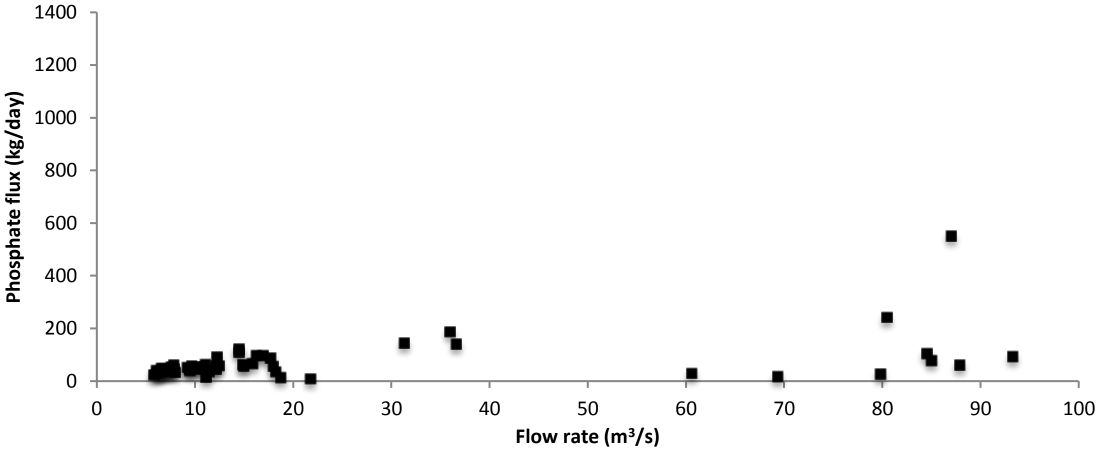


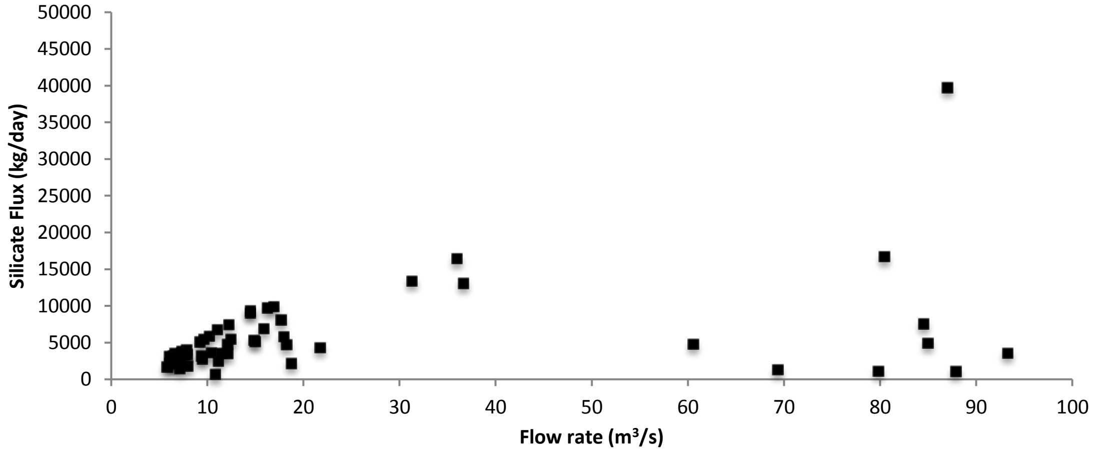
Appendix 3 – Relationships between River flux and inorganic nutrients

3.1 Knapp Mill

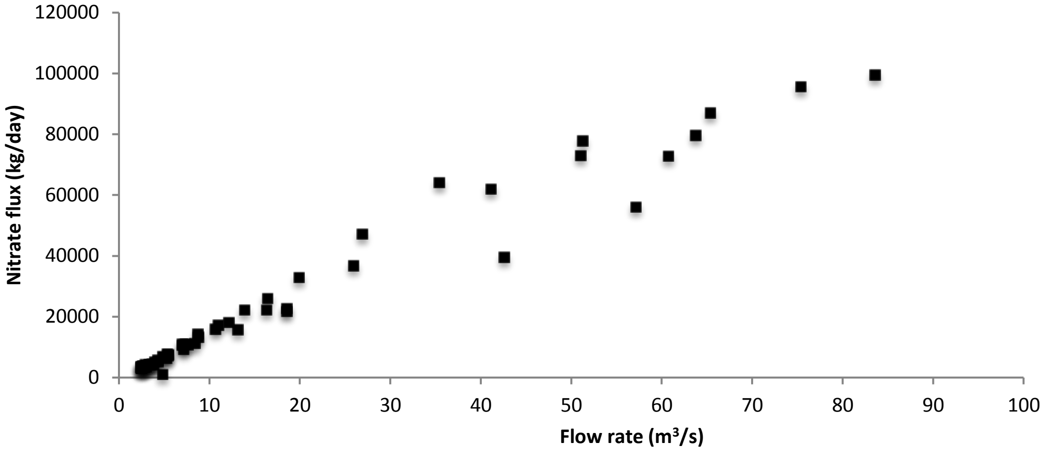


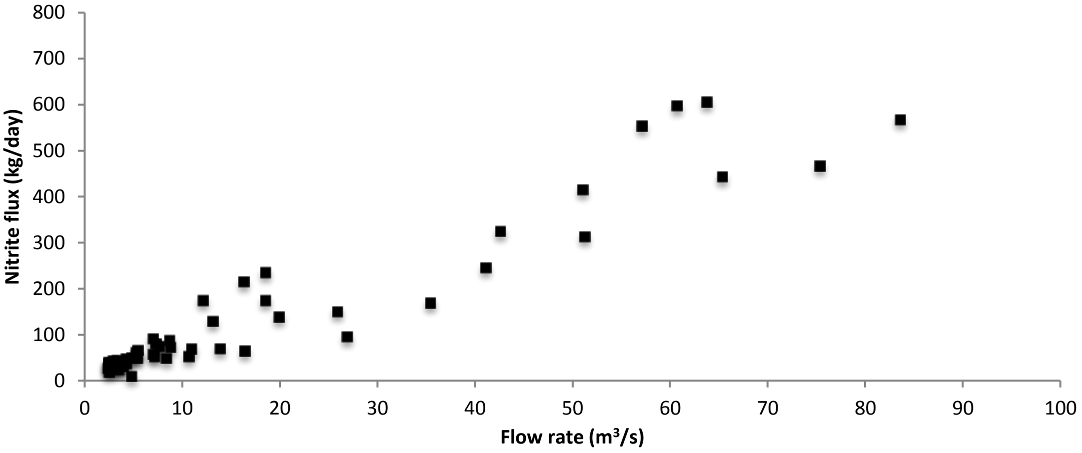


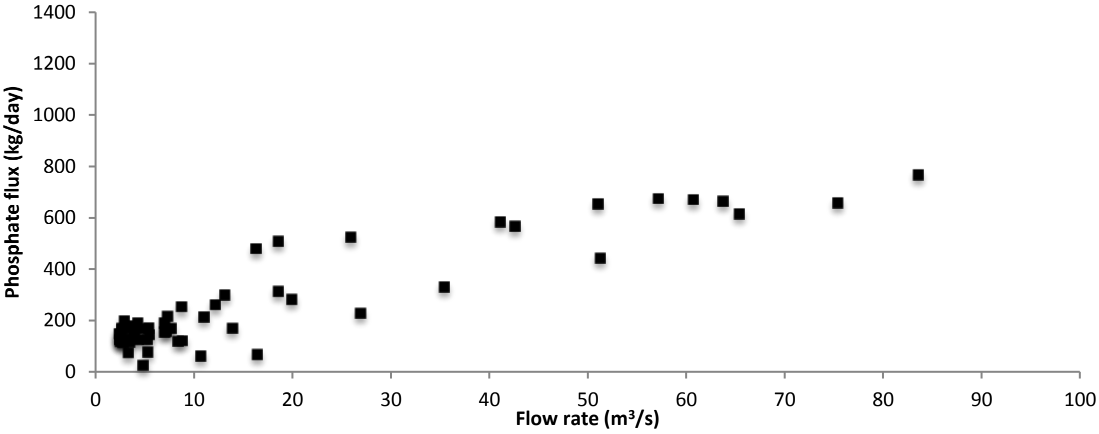


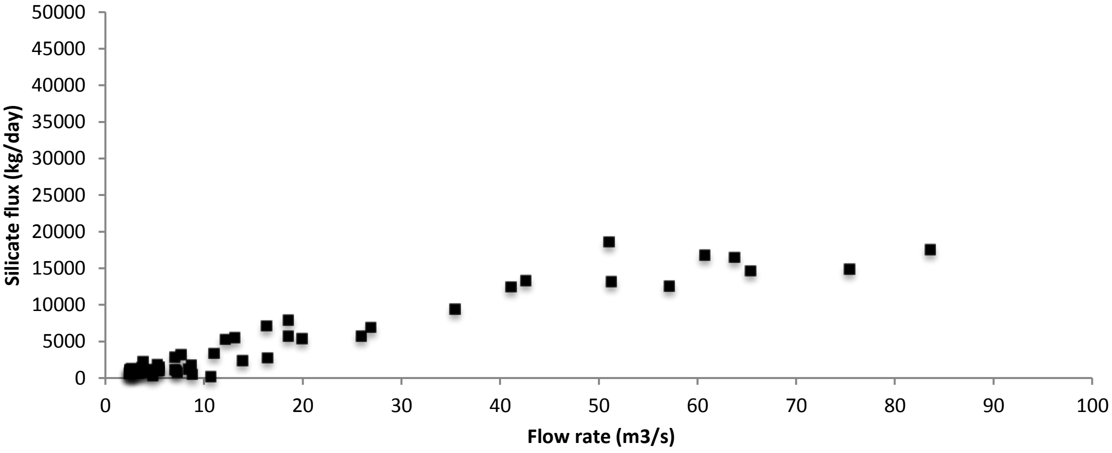


3.2 Throop


Nitrate flux (kg/day)
120000
100000
80000
60000
40000
20000
0
0
10
20
30
40
50
60
70
80
90
100
Flow rate (m³/s)


Nitrite flux (kg/day)
800
700
600
500
400
300
200
100
0
0
10
20
30
40
50
60
70
80
90
100
Flow rate (m³/s)


Phosphate flux (kg/day)
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
60
70
80
90
100
Flow rate (m³/s)


Silicate flux (kg/day)
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
0
10
20
30
40
50
60
70
80
90
100
Flow rate (m3/s)

List of references

- Abbyad, P., Tromp, J., Lam, J., & Salin, E. (2001). Optimization of the technique of standard additions for inductively coupled plasma mass spectrometry. *Journal of Analytical Atomic Spectrometry*, 16(5), 464-469.

- Ammerman, J. W., Hood, R. R., Case, D., & Cotner, J. B. (2003). Phosphorus deficiency in the Atlantic: an emerging paradigm in oceanography. *EOS*, 84, 165-170.

- Alexander, R. B., Smith, R. A., Schwarz, G. E., Boyer, E. W., Nolan, J. V,. & Brakebill, J.W. (2007). Differences in phosphorus and nitrogen delivery to the Gulf of Mexico from the Mississippi River Basin. *Environmental science & technology*, 42(3), 822-830.

- Armstrong, F. A. J., Stearns, C. R., & Strickland, J. D. H. (1967). The measurement of upwelling and subsequent biological process by means of the Technicon Autoanalyzer¨ and associated equipment. *Deep Sea Research and Oceanographic Abstracts*, 14(3), 381-389.

- Arreghini, S., de Cabo, L., Seoane, R., Tomazin, N., Seraf’ni, R., & de Iorio, A. F. (2005). Influence of rainfall on the discharge, nutrient concentrations and loads of a stream of the Pampa Ondulada (Buenos Aires, Argentina). *Limnetica*, 24(3-4), 225-236.

- Atlas, Z., Pasek, M., & Sampson, J. (2015). Phosphorus speciation by coupled HPLC-ICPMS: low level determination of reduced phosphorus in natural materials. *In EGU General Assembly Conference Abstracts*, 17, 7952.

- Baines, S. B., & Pace, M. L. (1991). The production of dissolved organic matter by phytoplankton and its importance to bacteria: patterns across marine and freshwater systems. *Limnology and Oceanography*, 36(6), 1078-1090.

- Baldwin, D. S. (2013). Organic phosphorus in the aquatic environment. *Environmental Chemistry*, 10(6), 439-454.

- Beaton, A. D., Cardwell, C. L., Thomas, R. S., Sieben, V. J., Legiret, F. E., Waugh, E. M., Statham, P. J., Mowlem, M. C. & Morgan, H. (2012). Lab-on-chip measurement of nitrate and nitrite for in situ analysis of natural waters. *Environmental science & technology*, 46(17), 9548-9556.

- Bell, R. G. (1994). Behaviour of dissolved silica , and estuarine / coastal mixing and exchange processes at Tairua Harbour, New Zealand. *New Zealand Journal of Marine and Freshwater Research*, 28(1), 55-68.

- Benitez-Nelson, C. R. (2000). The biogeochemical cycling of phosphorus in marine systems. *Earth-Science Reviews*, 51(1-4), 109-135.

- Benitez-Nelson, C. R., & Buesseler, K. O. (1999). Variability of inorganic and organic phosphorus turnover rates in the coastal ocean. *Nature*, 398, 502-505.

- Benson, R. L., McKelvie, I.D., Hart, B. T., Troung, Y. B., & Hamilton, I. C. (1996). Determination of total phosphorus in waters and wastewaters by on-line UV/thermal induced digestion and flow injection analysis. *Analytica Chemica Acta*, 326, 29-39.

- Berman, T., & Bronk, D. A. (2003). Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems. *Aquatic Microbiology Ecolology*, 31(3), 279- 305.

- Bernard, C. Y., Laruelle, G. G., Slomp, C. P., & Heinze, C. (2009). Impact of changes in river nutrient fluxes on the global marine silicon cycle: a model comparison. *Biogeosciences Discussions*, 6(2), 4463-4492.

- Beusen, A. H. W., Bouwman, A, F., Durr, H, H., Dekkers, A, L, M., & Hartmann, J. (2009). Global patterns of dissolved silica export to the coastal zone: Results from a spatially explicit global model. *Global Biogeochemistry Cycles*, 23(4) GBA 02.

- Bieroza, M. Z., & Heathwaite, A.L. (2016). Unraveling organic matter and nutrient biogeochemistry in groundwater-fed rivers under baseflow conditions: uncertainty in in situ high-frequency analysis. *Science of the Total Environment*. 572, 1520-1533.

- Bondoc, K. G. V., Heuschele, J., Gillard, J., Vyverman, W., & Pohnert, G. (2016). Selective silicate-directed motility in diatoms. *Nature communications*, 7, 10540.

- Bothwell, M. L. (1985). Phosphorus limitation of lotic periphyton growth rates: An intersite comparison using continuous_flow troughs (Thompson River system, British Columbia). *Limnology and oceanography*, 30(3), 527-542.

- Bowen, J. L., & Valiela, I. (2001). The ecological effects of urbanization of coastal watersheds: historical increases in nitrogen loads and eutrophication of Waquoit Bay estuaries. *Canadian Journal of Fisheries and Aquatic Sciences*, 58(8), 1489-1500.

- Bowes, M. J., Jarvie, H. P., Halliday, S. J., Skeffington, R. A., Wade, A. J., Loewenthal, M., & Palmer-Felgate, E. J. (2015). Characterising phosphorus and nitrate inputs to a rural river using high-frequency concentration-flow relationships. *Science of the Total Environment*. 511, 608-620.

- CaBA, 2016. *http://www.catchmentbasedapproach.org/south-west/hampshire-avon, (21/9/16).*

- Cai, Y., & Guo, L. (2009). Abundance and variation of colloidal organic phosphorus in riverine, estuarine, and coastal waters in the northern Gulf of Mexico. *Limnology and Oceanography*, 54(4), 1393-1402.

- Calijuri, M. C., Cunha, D. G. F., Queiroz, L. A., Moccellin, J., & Miwa, A. C. P. (2008). Nutrients and chlorophyll-a concentrations in tropical rivers of Ribeira de Iguape Basin, SP, Brazil. *Acta Limnologica Brasiliensia*, 20(2), 131-138.

- Cameron, W.M., & Pritchard, D.W. (1963). Estuaries. In: M.N. Hill (Editor), The Sea. *Wiley Interscience, New York*. 2,306-324

- Cantarero, A., Lopez, M. B., Mah'a, J., Maestro, M. A., & Paz, A. (2002). Determination of Total and Dissolved Phosphorus in Agricultural Runoff Samples By Inductively Coupled Plasma Mass

Spectrometry. *Communications in Soil Science and Plant Analysis*, 33(15-18), 3431-3436. http://doi.org/10.1081/CSS-120014536

- Carpenter, S. R., Caraco, N. F., Correll, D. L., Howarth, R. W., Sharpley, A. N., & Smith, V.H. (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological applications*, 8(3), 559-568.

- CEC (2000). Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of Water Policy. *In: Communities CoE*, editor. L327, pp. 73.

- Cheatham, M. M., Sangrey, W. F., & White, W. M. (1993). Sources of error in external calibration ICP-MS analysis of geological samples and an improved non-linear drift correction procedure. *Spectrochimica Acta Part B: Atomic Spectroscopy*, 48(3), 487-506. http://doi.org/10.1016/0584-8547(93)80054-X

- Chester, R. (2009). Marine geochemistry. *John Wiley & Sons*.

- Chrost, R. J. & Overbeck, J. (1987). Kinetics of alkaline phosphaase ac- tivity and phosphorus availability for phytoplankton and bacteri- oplankton in Lake Plussee (North-German eutrophic lake). *Microbial Ecology*, 13, 229-248.

- Clark, L. L., Ingall, E. D., & Benner, R. (1998). Marine phosphorus is selectively remineralized. *Nature*, 393(6684), 426.

- Collos, Y. (1998). Nitrate uptake, nitrite release and uptake, and new production estimates. *Marine Ecology Progress Series*. 171, 293-301.

- Conley, D. J., & Malone, T. C. (1992). Annual cycle of dissolved silicate in Chesapeake Bay: implications for the production and fate of phytoplankton. *Marine Ecology Progress Series*, 121-128.

- Conley, D. J., Schelske, C. L., & Stoermer, E. F. (1993). Modification of the Biogeochemical Cycle of Silica with Eutrophication. *Marine Ecology Progress Series*, 101, 179-192.

- Cooper, J., Lombardi, R., Boardman, D., & Carliell-Marquet, C. (2011). The future distribution and production of global phosphate rock reserves. *Resources, Conservation and Recycling*, 57, 78-86.

- Corbett, D. R. (2010). Resuspension and estuarine nutrient cycling: insights from the Neuse River Estuary. *Biogeosciences*, 7, 3289-3300.

- Corey, J, M., & Caruso, J, A. (1992). Electrothermal vaporization for sample introduction in plasma source spectrometry. *Critical reviews in Analytical Chemistry*, 23(5), 397-439.
-
- Correll, D. L. (1999). Phosphorus: a rate limiting nutrient in surface waters. *Poultry Science*, 78(5), 674-682.

- Council of the European Union, (2012) Water quality: restriction of phosphates in household detergents. Press release 6310/12.

http://www.consilium.europa.eu/uedocs/cms_data/docs/pressdata/en/intm/127928.pdf, *(Accessed 21/9/16).*

- Crump, B.C., Hopkinson, C.S., Sogin, M.L. & Hobbie, J.E. (2004). Microbial biogeography along an estuarine salinity gradient: combined influences of bacterial growth and residence time. *Applied and environmental microbiology*, 70(3), 1494-1505.

- Couceiro, F., Fones, G. R., Statham, P. J., Kelly-Gerreyn, B.A., Sivyer, D.B., Thompson, C. E. L., Parker R., and Amos C. L. (2013). Impact of resuspension of cohesive sediments at the Oyster Grounds (North Sea) on nutrient exchange across the sediment-water interface. *Biogeochemistry*, 113, 37-52.

- Daniel, M. H., Montebelo, A. A., Bernardes, M. C., Ometto, J. P., De Camargo, P. B., Krusche, A. V., Ballester, M. V., Victoria, R. L., & Martinelli, L. A. (2002). Effects of urban sewage on dissolved oxygen, dissolved inorganic and organic carbon, and electrical conductivity of small streams along a gradient of urbanization in the Piracicaba river basin. *Water, Air, and Soil Pollution*, 136(1-4), 189-206.

- Dayton, E. A., Whitacre, S., & Holloman, C. (2017). Comparison of three persulfate digestion methods for total phosphorus analysis and estimation of suspended sediments. Applied *Geochemistry*, 78, 357-362.

- DEFRA, (2002) Sewage Treatment in the UK - UK Implementation of the EC Urban Waste Water Treatment Directive, DEFRA Publication.s *https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/69582/pb6655-uk-sewage-treatment-020424.pdf, (Accessed 21/9/16).*

- DEFRA, (2005). Reform of the regulatory system to control small sewage discharges from septic tanks and small sewage treatment plants in England General - binding rules for small sewage discharges (SSDs) with effect from January 2015. DEFRA Publications *https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/397173/ssd-general-binding-rules.pdf, (Accessed 22/9/16).*

- Demars, B., & Harper, D. M. (1998). The aquatic macrophytes of an English lowland river system: assessing response to nutrient enrichment. *Hydrobiology*, 384(1-3), 75-88.

- Denison, F. H., Haygarth, P. M., House, W. A., & Bristow, A. W. (1998). The Measurement of Dissolved Phosphorus Compounds: Evidence for Hydrolysis During Storage and Implications for Analytical Definitions in Environmental Analysis. International Journal of Environmental *Analytical Chemistry*, 69(2), 111-123.

- Dettmann, E.H. (2001). Effect of water residence time on annual export and denitrification of nitrogen in estuaries: a model analysis. *Estuaries*, 24(4), 481-490.

- Dham, V. V., Wafar, M., & Heredia, A. M. (2005). Nitrogen uptake by size-fractionated phytoplankton in mangrove waters. *Aquatic Microbial Ecology*, 41(3), 281-291.

- Diaz, J., Ingall, E., Benitez-Nelson, C., Paterson, D., de Jonge, M., McNulty, I., & Brandes, J. A. (2008). Marine Polyphosphate: A Key Player in Geologic Phosphorus Sequestration. *Science*, 320(5876), 652-55.

- Dippner, J. W. (1998). Competition between different groups of phy- toplankton for nutrients in the southern North Sea. *Journal of Marine Systems*. 14. 181-198.

- Doney, S.C., Fabry, V.J., Feely, R.A., & Kleypas, J.A. (2009). Ocean acidification: The other CO2 problem. *Marine Science*, 1, 169-192.

- Dong, L. F., Sobey, M. N., Smith, C. J., Rusmana, I., Phillips, W., Stott, A., & Nedwell, D. B. (2011). Dissimilatory reduction of nitrate to ammonium, not denitrification or anammox, dominates benthic nitrate reduction in tropical estuaries. *Limnology and Oceanography*, 56(1), 279-291.

- Drever, J. I. (1997) The geochemistry of natural waters:surface and groundwater environments. *Lebanon USA: Prentice Hall.*

- Durand, P., Breuer, L., Johnes, P. J., Billen, G., Butturini A., Pinay, G., Van Grinsven. H. (2011). Nitrogen processes in aquatic ecosystems. In: Sutton MA, editor. The European nitrogen assessment: sources, effects, and policy perspectives. *Cambridge: Cambridge University Press*. 126-146.

- Dyhrman, S. T., Chappell, P. D., Haley, S. T., Moffett J. W, Orchard E. D., Waterbury J. B., & Webb E. A. (2006). Phosphonate utilization by the globally important marine diazotroph Trichodesmium. *Nature*, 439(7072), 68-71.

- Dyhrman, S. T., & Ruttenberg, K. C. (2006). Presence and regulation of alkaline phosphatase activity in eukaryotic phytoplankton from the coastal ocean: Implications for dissolved organic phosphorus remineralization. *Limnology and Oceanography*, 51(3), 1381-1390.

- Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., & Smith, J. E. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters*, 10(12), 1135-1142.

- Ekman, R. (2008). Mass spectrometry: instrumentation, interpretation, and applications. *John Wiley & Sons, Hoboken, NJ.*

- EPA. (2012). 5.9 Conductivity. Water: Monitoring and Assessment. Retrieved from *http://water.epa.gov/type/rsl/monitoring/vms59.cfm, (Accessed 21/9/16).*

- Fang, T. H. (2000). Partitioning and Behaviour of Different Forms of Phosphorus in the Tanshui Estuary and One of its Tributaries, Northern Taiwan. Estuarine, Coastal and Shelf Science, 50(5), 689-701.

- Ferreira, J. G., Andersen, J. H., Borja, A., Bricker, S. B., Camp, J., Cardoso da Silva, M., & Claussen, U. (2011). Overview of eutrophication indicators to assess environmental status within the European Marine Strategy Framework Directive. *Estuarine, Coastal and Shelf Science*, 93(2), 117-131.

- Foley, B., Jones, I. D., Maberly, S. C., & Rippey, B. (2012). Long_term changes in oxygen depletion in a small temperate lake: effects of climate change and eutrophication. *Freshwater Biology*, 57(2), 278-289.

- Fugate, D.C., & Friedrichs, C.T. (2003). Controls on suspended aggregated size in partially mixed estuaries. *Estuarine Coastal and Shelf Science*, 58, 389–404.

- Galloway, J. N., Dentener, F. J., Capone, D. G., Boyer, E. W., Howarth, R. W., Seitzinger, S. P., Asner, G. P., Cleveland, C. C., Green, P. A., Holland, E. A., Karl, D. M., Michaels, A. F., Porter, J. H., Townsend, A. R., & Vorosmarty, C. J. (2004). Nitrogen cycles past, present, and future. *Biogeochemistry*, 70, 153-226.

- Giblin, A. E., Weston, N., Banta, G., Tucker, J., & Hopkinson, C. S. (2010) The effects of salinity on nitrogen loss from an oligohaline estuarine sediment. *Estuaries and Coasts*, 33, 1054-1068.

- Gilmartin, M. (1967). Changes in inorganic phosphate concentration occurring during seawater sample storage. *Limnology and Oceanography*, 12(2), 325-328.

- Gypens, N., Delhez, E., Vanhoutte-Brunier, A., Burton, S., Thieu, V., Passy, P., & Lancelot, C. (2013). Modelling phytoplankton succession and nutrient transfer along the Scheldt estuary (Belgium, The Netherlands). *Journal of Marine Systems*, 128, 89-105.

- Han, C., Gu, X., Geng, J., Hong, Y., Zhang, R., Wang, X., & Gao, S. (2010). Production and emission of phosphine gas from wetland ecosystems. *Journal of Environmental Sciences*, 22(9), 1309-1311.

- Harrison, P. J., Hu, M. H., Yang, Y.P., & Lu, X., (1990). Phosphate limitation in estuarine and coastal waters of China. *Journal of Experimental Marine Biology and Ecology*, 140(1), 79-87.

- Heathwaite, A. L. (2010). Multiple stressors on water availability at global to catchment scales: Understanding human impact on nutrient cycles to protect water quality and water availability in the long term. *Freshwater Biology*, 55, 241-257.

- Heerboth, M. (2007). Speciation of Organic Phosphorus in Soils and Surface Waters by Liquid Chromatography with High Resolution Mass Spectrometry Detection., *Ph.D Thesis, Florida State University, Tallahassee.*

- Henriksen, A. A., & A. R. Selmer-Olsen. (1970). Automatic methods for determining nitrate and nitrite in water and soil extracts. *Analyst*, 95(1130), 514-518.

- Herman, J. S., Mills A. L., Hornberger G. M., A. C. Sofranko A. C., & Olson M. S. (2008) Quantifying Nitrate Flux during Storm Events. *In World Environmental and Water Resources Congress 2008*, 1-5.

- Holmes, R. M., McClelland, J. W., Peterson, B. J., Tank, S. E., Bulygina, E., Eglinton, T. I., Gordeev, V. V., Gurtovaya, T. Y., Raymond, P. A., Repeta, D. J., & Staples, R. (2012). Seasonal and annual fluxes of nutrients and organic matter from large rivers to the Arctic Ocean and surrounding seas. *Estuaries and Coasts*, 35(2), 369-382.

- Hoppe, H. G. (2003). Phosphatase activity in the sea. *Hydrobiologia*, 493, 187-200.

- Howarth, R. W., & Marino, R. (2006). Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: evolving views over three decades. *Limnology and Oceanography*, 51(1), 364-37.

- Howarth, R., Chan, F., Conley, D.J., Garnier, J., Doney, S.C., Marino, R. & Billen, G. (2011). Coupled biogeochemical cycles: eutrophication and hypoxia in temperate estuaries and coastal marine ecosystems. *Frontiers in Ecology and the Environment*, 9(1), 18-26.

- Howden, N. J. K., & Burt, T.P. (2009). Statistical analysis of nitrate concentrations from the Rivers Frome and Piddle (Dorset, UK) for the period 1965-2007. *Ecohydrology*, 2(1), 55-65.

- Huang, B., & Hong, H. (1999). Alkaline phosphatase activity and utilization of dissolved organic phosphorus by algae in sub- tropical coastal waters. *Marine Pollution Bulletin*, 19(1-12), 205-211.

- Huijts, K. M. H., Schuttelaars, H. M., Swart, H. E., & de Valle-Levinson, A. (2006). Lateral entrapment of sediment in tidal estuaries: an idealized model study. *Journal of Geophysical Research*, 111, 14.

- Human, L. R. D., Snow, G. C., Adams, J. B., & Bate, G. C. (2015). The benthic regeneration of N and P in the Great Brak Estuary. *Water* SA, 41, 594–605.

- Humborg, C., Conley, D.J., Rahm, L., Wulff, F., Cociasu, A., & Ittekkot, V. (2000). Silicon retention in river basins: far-reaching effects on biogeochemistry and aquatic food webs in coastal marine environments. *AMBIO: A Journal of the Human Environment*, 29(1), 45-50.

- Islam, M. J., Jang, C., & Eum, J. (2013). The decomposition rates of organic phosphorus and organic nitrogen in river waters, *Journal of Freshwater Ecology*, 28(2), 239-250.

- Ivanov, K., Zaprjanova, P., Angelova, V., Bekjarov, G., & Dospatliev, L. (2010). ICP determination of phosphorous in soils and plants. *19th World Congress of Soil Science, Soil Solutions for a Changing World*, 71-74.

- Jarvie, H. P., Neal, C., & Withers, P. J. (2006). Sewage-effluent phosphorus: a greater risk to river eutrophication than agricultural phosphorus? *Science of the Total Environment*, 360(1-3), 246-53.

- Jarvie, H., Withers, P., & Neal, C. (2002a). Review of robust measurement of phosphorus in river water: sampling, storage, fractionation and sensitivity. *Hydrology and Earth System Sciences*, 6(1), 113-131.

- Jarvie, H. P., Neal, C., Warwick, A., White, J., Neal, M., Wickham, H. D., & Andrews, M. C. (2002b). Phosphorus uptake into algal biofilms in a lowland chalk river. *Science of the Total Environment*, 282, 353-373.

- Jarvie, H.P., Neal, C., Withers, P.J., Wescott, C., & Acornley, R.M. (2005). Nutrient hydrochemistry for a groundwater-dominated catchment: the Hampshire Avon, UK. *Science of the Total Environment*, 344(1), 143-158.

- Jarvie, H.P., Neal, C., Withers, P.J.A., Robinson, A., Salter, N. (2003). Nutrient water quality of the Wye catchment, UK: exploring patterns and fluxes using the Environment Agency data archives. *Hydrology and Earth Systems Sciences*, 7, 722-743.

- Jickells, T. D., Andrews, J., Parkes, D., Suratman, S., Aziz, A., & Hee, Y. (2014). Nutrient transport through estuaries: the importance of the estuarine geography. *Estuarine Coastal and Shelf Science*, 150(B), 215–219.

- Karl, D., & Bjorkman, K. M. (2001). Phosphorus cycle in seawater: dissolved and particulate pool inventories and selected phosphorus fluxes. *Methods in Microbiology*, 30, 239-270.

- Kattner, G. (1999). Storage of dissolved inorganic nutrients in seawater: Poisoning with mercuric chloride. *Marine Chemistry*, 67(1-2), 61-66.

- Kolowith, L. C., Ingall, E. D., & Benner, R. (2001). Composition and cycling of marine organic phosphorus. *Limnology and Oceanography*, 46(2), 309-320.

- Kotlash, A. R., & Chessman, B. C. (1998). Effects of water sample preservation and storage on nitrogen and phosphorus determinations: implications for the use of automated sampling equipment. *Water Research*, 32(12), 3731-3738

- Lambert, D., & Maher, W. (1995). An evaluation of the efficiency of the alkaline persulfate digestion method for the determination of total phosphorus in turbid waters. *Water Research*, 29(1), 7-9.

- Lambert, D., Maher, W.A. & Hogg, I. (1992). Changes in phosphorus fractions during storage of lake water. *Water Research*, 26, 645-648.

- Lansdown, K., McKew, B. A., Whitby, C., Heppell, C. M., Dumbrell, A. J., Binley, A., Olde, L., & Trimmer, M. (2016). Importance and controls of anaerobic ammonium oxidation influenced by riverbed geology. *Nature Geoscience*, 9,(5), (357-360.

- Lin, P., Guo, L., Chen, M., & Cai, Y. (2013). Distribution, partitioning and mixing behavior of phosphorus species in the Jiulong River estuary. *Marine Chemistry*, 157, 93-105.

- Lindemann, T., Hinrichs, J., Hamester, M., & Wills, J. (2007). Simultaneous phosphorus and sulfur speciation by HPLC interfaced with high resolution ICP-MS. *Application Note*, 30076.

- Liu, S. M., Hong, G. H., Zhang, J., Ye, X. W.m & Jiang, X. L. (2009). Nutrient budgets for large Chinese estuaries. *Biogeosciences*, 6, 2245-63.

- Lloyd, C. E. M., Freer, J. E., Johnes, P. J., Coxon, G., & Collins, A.L. (2016). Discharge and nutrient uncertainty: implications for nutrient flux estimation in small streams. *Hydrological Processes,* 30(1), 135-152.

- Loken, L.C., Small, G.E., Finlay, J.C., Sterner, R.W., & Stanley, E.H. (2016). Nitrogen cycling in a freshwater estuary. *Biogeochemistry*, 127(2-3), 199-216.

- Lomas, M. W., & Lipschultz, F. (2006). Forming the primary nitrite maximum: Nitrifiers or phytoplankton? *Limnology and Oceanography*, 51(5), 2453-2467.

- Lomas, M. W., Burke, a. L., Lomas, D. a., Bell, D. W., Shen, C., Dyhrman, S. T., & Ammerman, J. W. (2010). Sargasso Sea phosphorus biogeochemistry: an important role for dissolved organic phosphorus (DOP). *Biogeosciences*, 7(2), 695-710.

- Magalhaes. C., Teixeira. C., Teixeira. R., Machado. A., Azevedo. I., & Bordalo. A. A. (2008). Dissolved organic carbon and nitrogen dynamics in the Douro River estuary, Portugal. *Cienc Marinas.* 34, 271-82.

- Maher, M., Forster, N., Krikowa, F., Snitch, P., Chapple, G., & Craig, P. (2001). Measurement of trace elements and phosphorus in marine animal and plant tissues by low-volume microwave digestion and ICP-MS. *Atomic Spectroscopy*, 22(5), 361-404.

- Maher, W., & Woo, L., (1998). Procedures for the storage and digestion of natural waters for the determination of filterable reactive phosphorus, total filterable phosphorus and total phosphorus. *Analytica Chimica Acta*, 375, 5-47.

- Mainstone, C. P., Holmes, N. T., Armitage, P. D., Wilson, A. M., Marchant, J. H., Evans, K., & Solomon, D. (1999). Chalk rivers-nature conservation and management. *Report to English Nature and the Environment Agency*, 184.

- Mainstone, C.P., & Parr, W. (2002). Phosphorus in rivers-ecology and management. *Science of the Total Environment*, 282, 25-47

- Majed, N., Li, Y., & Gu, A. Z. (2012). Advances in techniques for phosphorus analysis in biological sources. *Current Opinion in Biotechnology*, 23(6), 852-859.

- Malard, F., Tockner, K., Dole-Olivier, M-J., &Ward, J.V. (2002). A landscape perspective of surface–subsurface hydrological exchanges in river corridors. *Freshwater Biology*, 47, 621–640.

- Malone, T. C., Crocker, L. H., Pike, S. E., & Wendler, B. W. (1988). Influences of river flow on the dynamics of phytoplankton production in a partially stratified estuary. *Marine Ecology Progress Series*, 48(1982), 235-249. .

- Mantaser, A. (1998). Inductively Coupled Plasma Mass Spectrometry. *Wiley, New York*.

- Martiny, A. C., Kathuria, S., & Berube, P. M. (2009). Widespread metabolic potential for nitrite and nitrate assimilation among Prochlorococcus ecotypes. *Proceedings of the National Academy of Sciences of the United States of America*, 106(26), 10787-10792.

- Mather R. L., Reynolds S. E., Wolff G. A., Williams R. G., Torres-Valdes S., Woodward E. M. S., Landolfi A., Pan X., Sanders R., & Achterberg E. P. (2008). Phosphorus cycling in the North and South Atlantic Ocean subtropical gyres. *Nature Geoscience*, 1, 439-443.

- Matson, P. A., Parton. W. J., Power, A. G., & Swift, M. J. (1997). Agricultural intensification and ecosystem properties. *Science* 277, 504-509.

- Maya, K., Babu, K. N., Padmalal, D., & Seralathan, P. (2007). Hydrochemistry and dissolved nutrient flux of two small catchment rivers, south-western India. *Chemistry and Ecology*, 23(1), 13-27.

- McIsaac, G. F., David, M. B., Gertner, G. Z., & Goolsby, D.A. (2001). Eutrophication: Nitrate flux in the Mississippi river. *Nature*, 414(6860), 166-167.

- McKelvie, I. D., Peat, D. M., & Worsfold, P. J. (1995). Analytical perspective. Techniques for the quantification and speciation of phosphorus in natural waters. *Analytical Proceedings Including Analytical Communications*, 32(10), 437-445.

- McLusky, D. S., & Elliott, M. (2004). The estuarine ecosystem: Ecology, threats and management. *Oxford: Oxford University Press, 3rd edition*.

- Middelburg, J.J., & Herman, P. M. J. (2007). Organic matter processing in tidal estuaries. *Marine Chemistry*, 106(1–2), 127–147.

- Moore, W. S., Sarmiento, J. L., & Key, R. M. (2008). Submarine groundwater discharge revealed by Ra-228 distribution in the upper Atlantic Ocean. *Nature Geoscience*, 1, 309–11.

- Monaghan, E. J., & Ruttenberg, K. C. (1999). Dissolved organic phosphorus in the coastal ocean: Reassessment of available methods and seasonal phosphorus profiles from the Eel River Shelf. *Limnology and Oceanography*, 44(7), 1702-1714.

- Monbet, P., McKelvie, I. D., & Worsfold, P. J. (2009). Dissolved organic phosphorus speciation in the waters of the Tamar estuary (SW England). *Geochimica et Cosmochimica Acta*, 73(4), 1027-1038.

- MSFD, (2008) Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive). *Official journal of the European Union*, 51.

- Mulholland, P. J., & Hill, W. R. (1997). Seasonal patterns in streamwater nutrient and dissolved organic carbon concentrations: separating catchment flow path and instream effects. *Water Resource Research*, 33(6), 1297-1306.

- Murphy, J, A., & Riley, J, P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica chimica acta*, 27, 31-6.

- Muylaert, K., Sabbe, K., & Vyverman, W. (2000). Spatial and temporal dynamics of phytoplankton communities in a freshwater tidal estuary. *Estuarine, Coastal and Shelf Science*. 50(5), 673-687.

- Nausch, M., & Nausch, G. (2007). Bioavailable dissolved organic phosphorus and phosphorus use by heterotrophic bacteria. *Aquatic biology*, 1(2), 151-160.

- Neal, C., Jarvie, H. P., Howarth, S. M., Whitehead, P. G., Williams, R. J., Neal, M., & Wickham, H. (2000b). The water quality of the River Kennet: initial observations on a lowland chalk stream impacted by sewage inputs and phosphorus remediation. *Science of the Total Environment*, 251-252, 477-95.

- Neal, C., Jarvie, H. P., Williams, R. J., Neal, M., Wickham, H., & Hill, L. (2002). Phosphorus-calcium carbonate saturation relationships in a lowland chalk river impacted by sewage inputs and phosphorus remediation: An assessment of phosphorus self-cleansing mechanisms in natural waters. *Science of the Total Environment*, 282-283, 295-310.

- Neal, C., Jarvie, H., Whitton, B., & Gemmell, J. (2000a). The water quality of the River Wear, north-east England. *Science of the Total Environment*, 251-252, 153-72.

- Neal, C., Jarvie, H.P., Neal, M., Hill, L., & Wickham, H. (2006). Nitrate concentrations in river waters of the upper Thames and its tributaries. *Science of the Total Environment*, 365(1), 15-32.

- Nedwell, D. B., Jickells, T. D., Trimmer, M., & Sanders, R. (1999) Nutrients in estuaries. In: Fitter, A. H., Raffaelli, D. G., editors. *Advances in ecological research*, 29, 43–92.

- Nedwell, D. B., Dong, L. F., Sage, A. & Underwood, G. J. C. (2002). Variations of the nutrients loads to the mainland U.K. estuaries: correlation with catchment areas, urbanization and coastal eutrophication. *Estuarine, Coastal and Shelf Science*, 54, 951–970.

- Nielsen, E., & Richardson, K. (1996). Can changes in the fisheries yield in the Kattegat (1950-1992) be linked to changes in primary production? *Journal of Marine Science*. 53, 988-994.

- Nixon, S. W., Ammerman, J., & Atkinson, L. (1996). The fate of nitrogen and phosphorus at the land sea margin of the North Atlantic Ocean. *Biogeochemistry*, 35(1), 141-180.

- Nutrient Manangement Plan, (2009). Tried & Tested, Nutrient Management Plan. Professional nutrient management Group. Available: *https://www.gov.uk/guidance/fertiliser-recommendations-for-crops, (Accessed 5/9/16).*

- Olson, R. (1981). Differential photoinhibition of marine nitrifying bacteria: A possible mechanism for the formation of the primary nitrite maximum. *Journal of Marine Research*, 39, 227-238.

- Ormaza-Gonz‡lez, F.I., & Statham, P.J. (1996). A comparison of methods for the determination of dissolved and particulate phosphorus in natural waters. *Water Research*, 30(11), 2739-2747.

- Orr, J.C., Fabry, V.J., Aumont, O., Bopp, L., Doney, S.C., Feely, R.A., Gnanadesikan, A., Gruber, N., Ishida, A., Joos, F., & Key, R.M., (2005). Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature*, 437(7059), 681-686.

- Ostrofsky, M. L. (2012). Determination of total phosphorus in lake sediments. *Hydrobiologia*, 696(1), 199-203.

- Paytan, A., & McLaughlin, K. (2007). The oceanic phosphorus cycle. *Chemical Reviews*, 107(2), 563-76.

- Peat, D. M. W., McKelvie, I. D., Matthews, G. P., Haygarth, P. M., Worsfold, P. J. (1997). Rapid determination of dissolved organic phosphorus in soil leachates and runoff waters by flow injection analysis with on-line photo-oxidation. *Talanta*, 45, 47-55.

- Perez-Ruiz, T., Mart'nez-Lozano, C., Tomas, V., Mart'n, J. (2001). Flow-injection spectrofluorimetric determination of dissolved inorganic and organic phosphorus in waters using on-line photo-oxidation. *Analytica chimica acta*, 442, 147-153.

- Perillo, G.M., (1995). Geomorphology and sedimentology of estuaries. *Elsevier*, 53, 1-16.

- Philippart, C. J. M., CadŽe, G. C., van Raaphorst, W., & Riegman, R. (2000). Long-term phytoplankton-nutrient interactions in a shallow coastal sea: Algal community structure, nutrient budgets, and denitrification potential. *Limnology and Oceanography*, 45(1), 131-144.

- Pitkanen, H., & Tamminen, T. (1995). Nitrogen and phosphorus as production limiting factors in the estuarine waters of the eastern Gulf of Finland. *Marine Ecology Progress Series*, 129, 283-294.

- Prastka, K., Sanders, R., & Jickells, T. (1998). Has the role of estuaries as sources or sinks of dissolved inorganic phosphorus changed over time? Results of a Kd study. *Marine Pollution Bulletin*. 36, 718-728.

- Pretty, J. L., Hildrew, & A. G., Trimmer, M. (2006). Nutrient dynamics in relation to surface-subsurface hydrological exchange in a groundwater fed chalk stream. *Journal of Hydrology*, 330(1–2), 84–100.

- Pröfrock, D., Leonhard, P., Wilbur, S., & Prange, A. (2004). Sensitive, simultaneous determination of P, S, Cl, Br and I containing pesticides in environmental samples by GC hyphenated with collision-cell ICP-MS. *Journal of Analytical Atomic Spectrometry*, 19(5), 623-631.

- Ragueneau, O., Chauvaud, L., Moriceau, B., Leynaert, A., Thouzeau, G., Donval, A., Le Loc'h, F., & Jean, F. (2005). Biodeposition by an invasive suspension feeder impacts the biogeochemical cycle of Si in a coastal ecosystem (Bay of Brest, France), *Biogeochemistry*, 75, 19-41.

- Ragueneau, O., Conley, D. J., Leynaert, A., Longphuirt, S. N., & Slomp, C. P. (2006) Responses of coastal ecosystems to anthropogenic perturbations of silicon cycling. In: Ittekot V, Unger D, Humborg C, An NA, editors. *The silicon cycle. Washington: Island press*.

- Raymo, M. E. (1991). Geochemical evidence supporting T. C. Chamberlin's theory of glaciation, *Geology*, 19, 344-347.

- Reed, N, M., Cairns, R, O., & Hutton, R, C. (1994). Characterization of polyatomic ion interferences in inductively coupled plasma mass spectrometry using a high resolution mass spectrometer. *Journal of Analytical Atomic Spectrometry*, 9, 88-896.

- Regelink, I. C., Koopmans, G. F., van der Salm, C., Weng, L., & van Riemsdijk, W. H. (2013). Characterization of Colloidal Phosphorus Species in Drainage Waters from a Clay Soil Using Asymmetric Flow Field-Flow Fractionation. *Journal of Environment Quality*, 42(2), 464.

- Richards, S., Paterson, E., Withers, P. J., & Stutter, M. (2015). The contribution of household chemicals to environmental discharges via effluents: combining chemical and behavioural data. *Journal of environmental management*, 150, 427-434.

- Riemann, B., Simonsen, P., & Stensgaard, L. (1989). The carbon and chlorophyll content of phytoplankton from various nutrient regimes. *Journal of Plankton Research*, 11(5), 1037-1045.

- Rinker, K. R., & Powell, R. T. (2006). Dissolved organic phosphorus in the Mississippi River plume during spring and fall 2002. *Marine Chemistry*, 102(1), 170-179.

- Risgaard-Petersen, N. (2003). Coupled nitrification-denitrification in autotrophic and heterotrophic estuarine sediments: On the influence of benthic microalgae. *Limnology and Oceanography,* 48(1), 93-105

- Rivett, M. O., Buss, S. R., Morgan, P., Smith, J. W., & Bemment, C. D. (2008). Nitrate attenuation in groundwater: a review of biogeochemical controlling processes. *Water research*, 42(16), 4215-4232.

- Rodushkin, I., Ruth, T., & Klockare, D. (1998). Non-spectral interferences caused by a saline water matrix in quadrupole and high resolution inductively coupled plasma mass spectrometry. *Journal of Analytical Atomic Spectrometry*, 13(3), 159-166.

- Rofner, C., Sommaruga, R., & Teresa PŽrez, M. (2016). Phosphate and ATP uptake by lake bacteria: Does taxonomical identity matter? *Environmental Microbiology*, 18, 4782-4793.

- Roig, B., Gonzalez, C., & Thomas, O. (1999). Simple UV/UV-visible method for nitrogen and phosphorus measurement in wastewater. *Talanta*, 50(4), 751-758.

- Royer, T. V., David, M. B., & Gentry, L.E. (2006). Timing of riverine export of nitrate and phosphorus from agricultural watersheds in Illinois: Implications for reducing nutrient loading to the Mississippi River. *Environmental Science & Technology*, 40(13), 4126-4131.

- Ruttenberg, K. C. (2003). The global phosphorus cycle. In: Schlesinger WH, editor. Treatise on geochemistry, *Elsevier*, 8, 585-643.

- Ruttenberg, K. C., & Sulak, D. J. (2011). Sorption and desorption of dissolved organic phosphorus onto iron (oxyhydr) oxides in seawater. *Geochimica et Cosmochimica Acta*. 75(15), 4095-4112.

- Ruttenberg, K. C., & Dyhrman, S. T. (2012). Dissolved organic phosphorus production during simulated phytoplankton blooms in a coastal upwelling system. *Frontiers in Microbiology*, 31-12.

- Qin, C., Liu, H., Liu, L., Smith, S., Sedlak, D. L., & Gu, A. Z. (2015). Bioavailability and characterization of dissolved organic nitrogen and dissolved organic phosphorus in wastewater effluents. *Science of the Total Environment*, 511, 47-53.

- Schindler, D. W., Armstrong, F. A. J., Holmgren, S. K., & Brunskill. G. J. (1971) Eutrophication of Lake 227, Experimental Lakes Area, northwestern Ontario, by addition of phosphate and nitrate. *Journal of the Fisheries Board of Canada*, 28(11), 1763-1782.

- Schlesinger, W. H., Reckhow, K. H., & Bernhardt, E. S. (2006). Global change: The nitrogen cycle and rivers. *Water Resources Research*, 42, (3).

- Sebasti‡n, M., Pitta, P., Gonz‡lez, J. M., Thingstad, T. F., & Gasol, J. M. (2012). Bacterioplankton groups involved in the uptake of phosphate and dissolved organic phosphorus in a mesocosm experiment with P-starved Mediterranean waters, *Environmental Microbiology*, 14, 2334-2347.

- SEDAC. (2011). Coastal population indicator: data and methodology page. Socioeconomics and data and applications center. *http://sedac.ciesin.columbia.edu/es/csdcoastal. Html, 2011, (Accessed 18/8/16).*

- Seitzinger, S. P., Harrison, J. A., Dumont, E., Buesen, A. H. W., & Bouwman, A. F. (2005). Sources and delivery of carbon, nitrogen, and phosphorus to the coastal zone: an overview of Global NEWS models. *Global Biogeochemical Cycles*, 19(4), 1-11.

- Seitzinger, S. P., Mayorga, E., Bouwman, A. F., Kroeze, C., Beusen, A. H. W., Billen, G., Van Drecht, G., Dumont, E., Fekete, B.M., Garnier, J. & Harrison, J.A. (2010). Global river nutrient export: A scenario analysis of past and future trends. *Global Biogeochemical Cycles*, 24(4).

- Sharples, J., Middelburg J. J., Fennel, K., & Jickells, T. D. (2017), What proportion of riverine nutrients reaches the open ocean? *Global Biogeochemistry Cycles*, 31, 39-58.

- Sigleo, A. C., & Frick, W. E. (2003). Seasonal variations in river flow and nutrient concentrations in a northwestern USA watershed. In First interagency conference on research in the watersheds. *US Department of Agriculture*, 370-376.

- Silvennoinen, H., Hietanen, S., Liikanen, A., Stange, C.F., Russow, R., Kuparinen, J., & Martikainen, P.J. (2007). Denitrification in the river estuaries of the northern Baltic Sea. *AMBIO: A Journal of the Human Environment*, 36(2), 134-140

- Smith, S. V., Kimmerer, W. J., & Walsh, T. W., (1986). Vertical flux and biogeochemical turnover regulate nutrient limitation of net organic production in the North Pacific Gyre. *Limnology and Oceanography*, 31(1), 161-166.

- Soetaert, K., Middelburg, J. J., Heip, C., Meire, P., Van, S., Maris, T., & Van Damme, S. (2010). Long-term changes in dissolved inorganic nutrients in the heterotrophic Scheldt estuary. *Limnology*, 51(1), 409-423.

- Statham, P. J. (2012). Nutrients in estuaries--an overview and the potential impacts of climate change. *Science of the Total Environment*, 434, 213-27.

- Stepanauskas, R., J¿rgensen, N. O. G., Eigaard, O. R., Zvikas, A., Tranvik, L. J., Leonardson, L. (2002). Summer inputs of riverine nutrients to the Baltic Sea: Bioavailability and eutrophication relevance. *Ecological monographs*, 72(4) 579- 597.

- Stolpe, B., Guo, L., Shiller, A. M., & Hassellšv, M. (2010). Size and composition of colloidal organic matter and trace elements in the Mississippi River, Pearl River and the northern Gulf of Mexico, as characterized by flow field-flow fractionation. *Marine Chemistry*, 118(3-4), 119-128.

- Strickland J. D. H., & Parsons, T. R. (1972). A practical handbook of seawater analysis. *Fisheries Research Board of Canada, Ottawa Second Edition*, 167.

- Suksomjit, M., Nagao, S., Ichimi, K., Yamada, T., and Tada, K. (2009). Variation of Dissolved Organic Matter and Fluorescence Characteristics before, during and after Phytoplankton Bloom. *Journal of Oceanography*, 65(6), 835-846.

- Tan, S, H., & Horlick, H. (1986). Back- ground spectral features in inductively coupled plasma/mass spectrometry. *Applied Spectroscopy*, 40, 445-460.

- Teixeira, C., Magalhaes, C., Boaventura, R. A. R., & Bordalo, A. A. (2010) Potential rates and environmental controls of denitrification and nitrous oxide production in a temperate urbanized estuary. *Marine environmental research*, 70, 336-42.

- ThermoScientific. (2017). Triple Quadrupole ICP-MS or High Resolution ICP-MS? Which Instrument is Right for Me? from *https://www.thermofisher.com/search/results?query=triple+quadrupole+high+resolution+icp-ms&sort=&l1=&onlineOfferId=&persona=DocSupport, (Accessed 3/3/17).*

- Tilman, D., Fargione, J., Wolff, B., D'Antonio, C., Dobson, A., Howarth, R., Schindler, D., Schlesinger, W. H., Simberloff, D., & Swackhamer, D. (2001) Forecasting agriculturally driven global environmental change. *Science*, 292(5515), 281-284.

- Turner, A., & Millward G.E. (2002). Suspended particles: Their role in estuarine biogeochemical cycles. *Estuarine, Coastal and Shelf Sciences*, 55, 857-883.

- Tréguer, P., Nelson, D. M. van Bennekom, A. J., DeMaster, D. J., Leynaert, A., & Quéguiner, B. (1995). The silica balance in the world ocean: a reestimate. *Science* 268, 375-379.

- UKTAG, (2008). *UK Environmental Standards and Conditions (Phase 1).*

- UKTAG, (2013) *Phosphorus Standards in Rivers. Updated Recommendations, p. 13.*

- Uncles, R. J., Fraser, A. I., Butterfield, D., Johnes, P., & Harrod, T.R., (2002). The prediction of nutrients into estuaries and their subsequent behaviour: application to the Tamar and comparison with the Tweed, UK. *Nutrients and Eutrophication in Estuaries and Coastal Waters*, 239-250.

- Van Moorleghem, C., De Schutter, N., Smolders, E., & Merckx, R. (2013). The bioavailability of colloidal and dissolved organic phosphorus to the alga Pseudokirchneriella subcapitata in relation to analytical phosphorus measurements. *Hydrobiologia*, 709(1), 41-53.

- Vitousek, P. M., Abet, J., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W., Schlesinger, W. H., & Tilman, G. D. (1997). Human alteration of the global nitrogen cycle: Causes and consequences. *Ecological Applications*, 7, 737-750.

- Wada, E., & Hattori, A. (1971). Nitrite metabolism in the euphotic layer of the central North Pacific Ocean. *Limnology and Oceanography*, 16, 766-772

- Wang, Z. L., & Liu, C. Q., (2003). Distribution and partition of heavy metals between dissolved and acid-soluble fractions along a salinity gradient in Changjiang Estuary, eastern China. *Chemical Geology*, 202, 383– 396.

- Wang, Q., & Li, Y. (2010). Phosphorus adsorption and desorption behaviour on sediments of different origins. *Journal of Soils and Sediments*, 10, 1159-73.

- Wetz, M. S., & Wheeler, P.A. (2003). Production and partitioning of organic matter during simulated phytoplankton blooms. *Limnology and Oceanography*, 48(5), 1808-1817.

- White, A. F., & Blum, A. E. (1995). Effects of climate on chemical weathering in watersheds, *Geochimica et Cosmochimica Acta*, 59, 1729-1747.

- Whitehead, P. G., Jin, L., Crossman, J., Comber, S., Johnes, P. J., Daldorph, P., & Willows, R. (2014). Distributed and dynamic modelling of hydrology, phosphorus and ecology in the Hampshire Avon and Blashford Lakes: Evaluating alternative strategies to meet WFD standards. *Science of the Total Environment*, 481(1), 157-166.

- Whitehead, P. G., Jin, L., Crossman, J., Comber, S., Johnes, P. J., Daldorph, P., & Willows, R. (2014). Distributed and dynamic modelling of hydrology, phosphorus and ecology in the Hampshire Avon and Blashford Lakes: Evaluating alternative strategies to meet WFD standards. *Science of the Total Environment*, 481(1), 157-166.

- Williams, P. J. L. (1995). Evidence for the seasonal accumulation of carbon-rich dissolved organic material, its scale in comparison with changes in particulate material and the consequential effect on net C/N assimilation ratios. *Marine Chemistry*, 51, 17.

- Worrall, F., Jarvie, H. P., Howden, N. J. K., & Burt, T. P. (2016). The fluvial flux of total reactive and total phosphorus from the UK in the context of a national phosphorus budget: comparing UK river fluxes with phosphorus trade imports and exports. *Biogeochemistry*, 130(1-2), 31-51.

- Worsfold, P. J., Gimbert, L. J., Mankasingh, U., Ndukaku Omaka, O., Hanrahan, G., Gardolinski, P. C. F. C., Haygarth, P. M., Turner, B. L., Keith-Roach, M. J., & McKelvie, I. D. (2005). Sampling, sample treatment and quality assurance issues for the determination of phosphorus species in natural waters and soils. *Talanta*, 66(2), 273-293.

- Worsfold, P. J., Gimbert, L. J., Mankasingh, U., Omaka, O. N., Hanrahan, G., Gardolinski, P. C. F. C., & McKelvie, I. D. (2005). Sampling, sample treatment and quality assurance issues for the determination of phosphorus species in natural waters and soils. *Talanta*, 66(2), 273-93.

- Worsfold, P., McKelvie, I., & Monbet, P. (2016). Determination of phosphorus in natural waters: A historical review. *Analytica Chimica Acta*, 918, 8-20.

- Wu, J., Sunda, W., Boyle, E., & Karl, D. (2000). Phosphate depletion in the western North Atlantic Ocean. *Science*, 289, 759-762.

- Yates, C. A., & Johnes, P. J. (2013). Nitrogen speciation and phosphorus fractionation dynamics in a lowland Chalk catchment. *Science of the Total Environment*, 444, 466-479.

- Yücel, M., Beaton, A. D., Dengler, M., Mowlem, M. C., Sohl, F., & Sommer, S. (2015). Nitrate and nitrite variability at the seafloor of an oxygen minimum zone revealed by a novel microfluidic in-situ chemical sensor. *PloS one*, 10(7).

- Zhang, G., Liang, S., Shi, X. and Han, X., (2015). Dissolved organic nitrogen bioavailability indicated by amino acids during a diatom to dinoflagellate bloom succession in the Changjiang River estuary and its adjacent shelf. *Marine Chemistry*, 176, 83-95.

- Zurawsky, M. A., Robertson, W. D., Ptacek, C. J., & Schiff, S. L. (2004). Geochemical stability of phosphorus solids below septic system infiltration beds. *Journal of Contaminant Hydrology*, 73, 129-143.