Do fish farms impact the water quality of chalk streams?

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Abstract

It is largely understood that water quality in the UK has become increasingly contaminated by numerous point and diffuse pollutant sources. Fish farms can introduce a broad range of pollutants; however, the influence of PO_4^{3-} has been largely neglected in studies of UK chalk streams. Therefore, this study aimed to monitor changes in nutrient concentrations and water quality parameters upstream and downstream of a trout farm in the River Itchen, Hampshire. Reactive phosphorus and PO_4^{3-} were measured at two sites over 24-days using an ISCO automated bottle sampler and QuAAtro autoanalyser, whilst conductivity, dissolved oxygen and temperature were measured using a CTD probe. Concentrations of orthophosphate, ammoniacal nitrogen and dissolved oxygen from 2000-2022 were obtained from three EA sites. Nutrients were compared to HadUKP rainfall data and UKTAG WFD thresholds to assess the source and extent of nutrient loading. Over the 24-day field trial and 22-year EA archive, concentrations of reactive phosphorus increased 50-metres downstream by an average of $0.38mgL^{-1}$ and $0.014mgL^{-1}$ respectively, degrading water quality from 'high' to 'good' WFD status. Ammoniacal nitrogen, temperature and conductivity also increased by $0.165mgL^{-1}$, 0.23°C and $6.05\mu scm^{-1}$, whilst dissolved oxygen reduced by 25-30%. Each parameter exhibited significant diurnal variation due to food waste, faecal matter and effluent processing errors from the trout farm. A lack of correlation with rainfall suggests the influence of diffuse sources further upstream was relatively minimal. All parameters recovered 1500-metres downstream, suggesting nutrient loading was only of concern to water quality over short distances. However, previous research suggests these trends could still be detrimental to salmonids and macroinvertebrate communities. With chalk streams consistently failing to meet 'good' WFD targets, this study provides critical insight into the influence of nutrient loading from fish farms to inform future nutrient mitigation strategies, improve water quality and avoid ecological implications.

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List of Abbreviations

ABS	Automated bottle sampler
EA	Environment Agency
EA1-3	Environment Agency sampling sites
EU WFD	European Union Water Framework Directive
HadUKP	Met Office Hadley Centre UK Observations
N	Nitrogen
P	Phosphorus
PO_4^3	Phosphate
S1-2	ISCO sampling sites
SAC	Special Area of Conservation
SSSI	Site of Special Scientific Interest
STW	Sewage treatment works
TSS	Total suspended solids
UKTAG WFD	UK Technical Advisory Group on the Water Framework Directive

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1. Introduction

1.1. River Pollution

Water quality summarises the chemical, biological and physical health of a water body (Le Moal et al., 2019). In the UK, the water quality of rivers is considered essential for biodiversity, drinking water, recreation and aesthetics (Bowes et al., 2011). However, recent research suggests that rivers have become increasingly pressurised under a cumulative “chemical cocktail” of pollutants (Environmental Audit Committee, 2022, p.5). These include nutrients, persistent organic pollutants and emerging synthetic stressors such as plant protection products, pharmaceuticals and microplastics (Robinson et al., 2022).

Nutrients are considered the most prevalent pollutants in freshwater bodies. In particular, phosphate (PO_4^{3-}) is considered highly influential due to its high bioavailability and ability to stimulate eutrophication (Hilton et al., 2006; Clark et al., 2017). Consequently, numerous legislative policies have been introduced to monitor the ecological and physiochemical status of freshwater bodies relative to nutrient loading, including the EU Water Framework Directive (WFD) and UK Technical Advisory Group (UKTAG) on the WFD (UKTAG, 2013; WFD, 2017).

As a result, many government reports suggest water quality in the UK has improved substantially since 1990, with a 2.1% annual average reduction in aqueous PO_4^{3-} concentrations (Foy et al., 2007; Whelan et al., 2022). Nevertheless, in 2022, only 14% of UK water bodies achieved the target of ‘good’ WFD status set by Defra (2014, p.17). One explanation for this is the recent government funding cuts to the Environment Agency (EA), which has reduced the frequency of water quality monitoring to overlook pollutant permit breaches from individual point and diffuse sources (Fones et al., 2020).

1.2. Point and Diffuse Sources

Pollutants can be introduced to freshwater bodies by either point or diffuse sources. Point sources introduce pollutants directly from sewage treatment works (STW), industrial effluent and fish farms, whilst diffuse sources leach pollutants indirectly through surface or groundwater from agricultural and urban activity (Bowes et al., 2009). Whilst studies suggest STW and agriculture are the primary pollutant inputs in freshwater systems, there are several sources that have been somewhat neglected in the scientific literature (Casey et al., 1993; Jarvie et al., 2006; Bowes et al., 2015).

1.3. Fish Farms and Chalk Streams

One neglected point source of pollutants is freshwater fish farms. In aquaculture, veterinary drugs and antibiotics are commonly used to control disease outbreaks (Justino et al., 2016). Furthermore, growth rates are maximised using feed overstimulated with metal salts and phosphate (PO_4^{3-}). Contaminants then enter water bodies through direct effluent discharges or indirect food waste and faecal excretion (Bergheim & Brinker, 2003).

Figure 1 shows the location of fish farms around the UK. A large proportion of freshwater farms are located on chalk aquifer-fed rivers in southern England, including the Test and Itchen (Jennings et al., 2016). Chalk streams have naturally low concentrations of PO_4^{3-} ($<0.02mgL^{-1}$) within 'high' WFD status (Cox, 2008, p.15). However, recent studies suggest that an increasing proportion of diurnal, seasonal and annual PO_4^{3-} concentrations have breached this threshold (Palmer-Felgate et al., 2008; Bowes et al., 2011; Fones et al., 2020). Whilst these trends have largely been attributed to STW and agriculture, the influence of freshwater fish farms has been largely neglected, despite being proven as a critical point source in many European studies (Foy & Rossel, 1991; Bergheim & Brinker, 2003; Pajooh et al., 2016). Consequently, this study aimed to assess if freshwater fish farms are a comparable nutrient source by monitoring the change in nutrient concentrations and water quality parameters upstream and downstream of an unstudied fish farm in the River Itchen, Hampshire.

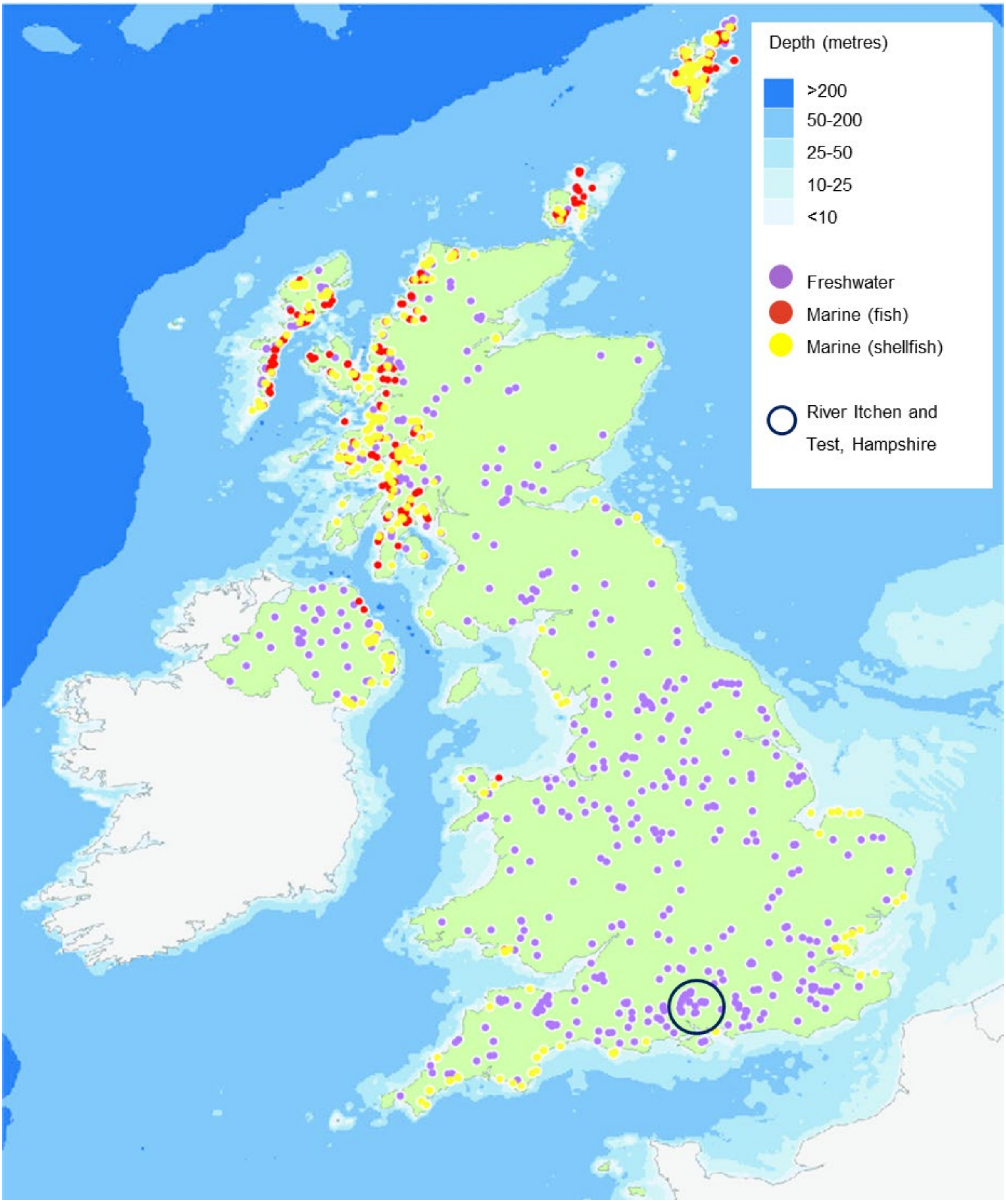


Figure 1 - Map of UK marine and freshwater fish farms, including the location of the Rivers Itchen and Test, Hampshire. Adapted from Jennings et al. (2016).

1.4. Project Location: Itchen Abbas

Figure 2 shows the location of the Itchen Abbas study area. The Itchen is a 45km chalk stream situated in Hampshire, UK (Figure 2a), with a mean discharge of 53m^3 s^{-1} and a base flow index of ~0.95 (Fones et al., 2020). The river flows through numerous rural areas, including Alresford and Itchen Abbas, before entering Winchester and Southampton (Figure 2b-c) (Casey & Smith, 1994). Therefore, the 400km^2 catchment encompasses a variety of pollutant sources, including agriculture, watercress farms and Itchen Abbas Trout Farm seen in Figure 2c, and STW further downstream. The river also supports a range of biodiversity, including species of macrophytes, mayflies and salmonids (WWF-UK, 2014).

The Itchen was selected for this study due to its minimal appearance in previous research, despite its protected status as a Site of Special Scientific Interest (SSSI) and Special Area of Conservation (SAC). Work by Casey & Smith (1994), Fones et al. (2020) and Robinson et al. (2022) form a limited study of the catchment, with pollution from watercress farms, STW and septic tanks resulting in failure to meet SAC targets. Therefore, a greater range of pollutant sources needs to be monitored to provide further insight into the water quality of this chalk stream.

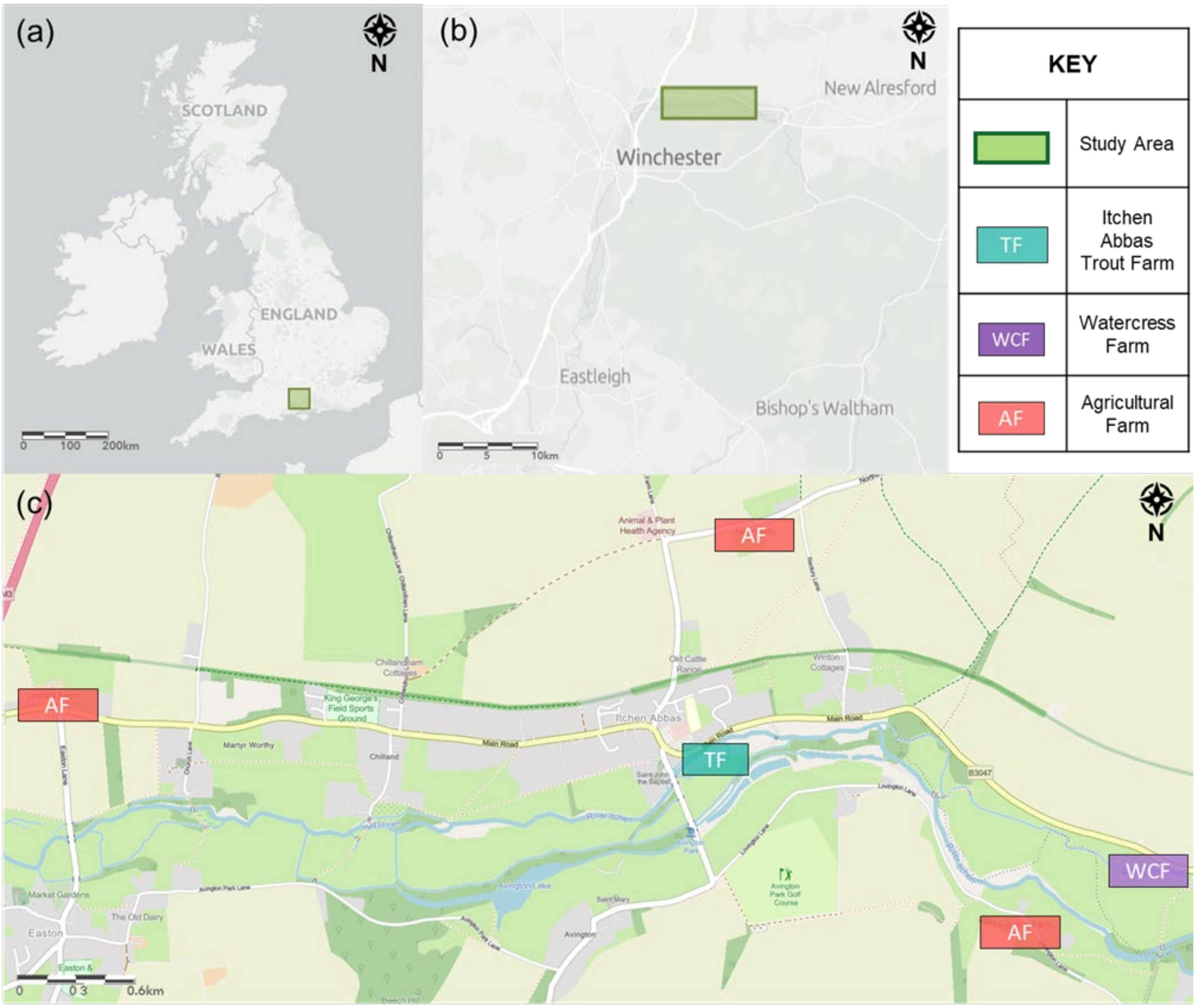


Figure 2. Map showing (a) the UK, (b) the study area and (c) Itchen Abbas with potential pollutant sources. From Ordinance Survey (2022a).

1.5. Aims and Objectives

This study aimed to assess the influence of Itchen Abbas Trout Farm on the water quality of the River Itchen. To achieve this, four primary objectives were established:

1. To measure diurnal concentrations of reactive phosphorus and PO_4^{3-} at two sites (a) upstream and (b) downstream of a fish farm over 24 days using high-frequency automated bottle sampling and compare differences statistically.
2. To analyse long-term trends in historic Environment Agency water quality data across the Itchen Abbas catchment at (a) Itchen Abbas Trout Farm Inlet, (b) Itchen Abbas Trout Farm Effluent and (c) Easton from 2000-2022, and statistically compare differences in parameters between sites.
3. To compare concentrations of reactive phosphorus sampled over the 24-day trial period and long-term data supplied by the Environment Agency to UKTAG WFD thresholds to assess the extent and status of nutrient pollution across the Itchen Abbas catchment.
4. To assess the influence of precipitation measured by the Met Office HADUKP on concentrations of reactive phosphorus sampled over the 24-day trial period and long-term trends in water quality parameters supplied by the Environment Agency by comparing relationships statistically.

1.6. Dissertation Structure

To gain a holistic understanding of water quality in chalk streams, a summary of research and policy was supplied in the ‘Literature Review’ (Chapter 2). The ‘Methodology’ (Chapter 3) outlines the field, laboratory and analytical approach to this study. ‘Results’ were presented and analysed in Chapter 4 and discussed with inference from the literature in the ‘Discussion’ (Chapter 5). Finally, the ‘Conclusion’ (Chapter 6) summarises the overall findings with a critical reflection on the study.

2. Literature Review

2.1. Water Quality Monitoring and Policies

Numerous government reports claim that average water quality in the UK is “better than at any time since the start of the Industrial Revolution” (Whelan et al., 2022, p.1). However, studies of regional freshwater bodies provide significant evidence to counteract this claim (Owens & Walling, 2002; Bowes et al., 2008; Goddard et al., 2020). This has highlighted the need to monitor spatial shifts of pollutants in different freshwater bodies, and how they influence water quality parameters over time.

Monitoring water quality requires the collection of physiochemical data (Massik & Costello, 1995). Temporal data can assist in identifying long-term changes, whilst spatial data offers insight into the sources responsible. The Environment Act (2021) propose a standardised list of physiochemical parameters to monitor, including ammonia, dissolved oxygen and temperature. Neal et al. (2000a) also recommend monitoring PO_4^{3-} and conductivity to provide a holistic perception of water quality. All these variables were included in this study. According to Carr & Goulder (1990), at least one site upstream and one site downstream of each pollution source should be included to compare shifts in pollutants and physiochemical parameters.

Legislative guidelines regarding the monitoring of water quality have also been established under the EU WFD (2017). This directive annually categorises water bodies as ‘high’, ‘good’, ‘poor’ or ‘bad’ based on numerous ecological and physiochemical parameters. Additional legislation related to PO_4^{3-}, alkalinity and altitude has been established by the UKTAG WFD, as seen in Appendix A (UKTAG, 2013). Nevertheless, most surface water in the UK has failed to achieve the target of ‘good’ WFD status, with 77% of chalk streams assessed as ‘poor’ or ‘bad’ in 2022 (Ball et al., 2022; van Kats et al., 2022). Consequently, the Environment Act (2021) have recently proposed ambitions to monitor water quality upstream and downstream of every pollution input site. Therefore, all pollution sources will be holistically monitored to inform and improve future management of water quality in rivers, wetlands and chalk streams around the UK.

2.2. The Importance of Water Quality in Chalk Streams

Chalk streams are considered to have an exceptional standard of water quality due to cumulative physical, chemical and geological factors, as discussed by Westlake et al. (1972). The porous chalk geology of chalk streams allows precipitation to directly percolate into the water table with minimal contamination from surface run-off, sediment and organic matter. According to Robinson et al. (2022), chalk aquifers also regulate the volume of water supplied to chalk streams, resulting in stable flow regimes and physiochemical parameters, including:

- A high alkalinity (>50mgL^{-1} CaCO3)
- A pH of 7.4-8
- A narrow temperature range (5-17°C).

Consequently, studies by Mann et al. (2006) and Larsen et al. (2011) suggest chalk streams support a biodiverse community of macrophyte, invertebrate and salmonid species that rely on these stable parameters, including blunt-fruited water-starwort (*Callitriche obtusangula*), wild brown trout (*Salmo trutta*) and the blue-winged olive (*Serratella ignita*). Furthermore, the natural stability and purity of chalk streams attract a range of anthropogenic uses, including watercress farming, recreational fishing and drinking water abstraction, and contribute significant aesthetic value to the local environment (WWF-UK, 2014). 80% of chalk streams are located in England; therefore, it is critical to monitor and regulate the water quality of these rare ecosystems relative to a range of pollution sources to maintain ecological communities, ecosystem services, recreational uses and aesthetic value (Bowes et al., 2008).

2.3. Nutrient Pollution in Chalk Streams

The water quality of chalk streams is vulnerable to a variety of pollutants. Studies by Fones et al. (2020) and Robinson et al. (2022) have identified a significant range of pollutant types in the Rivers Itchen and Test in Hampshire. These include plant protection products, persistent organic pollutants, pharmaceuticals and nutrients from inputs including STW, agriculture and urban run-off.

Phosphorus (P) and nitrogen (N) are naturally occurring, limiting nutrients essential for growth in terrestrial and marine organisms (Clark et al., 2017; Worsfold et al., 2016). Whilst numerous fractions of each nutrient are present in freshwater systems, dissolved (<0.02-0.45μm) inorganic PO_4^{3-} is considered the most critical nutrient pollutant due to its bioavailability to primary producers and potential to stimulate eutrophication and subsequent stress to ecological communities, as discussed in Sections 2.4 and 2.5 (Maruo et al., 2016; Cao et al., 2020).

Most chalk streams in the UK are occupied by either a point or diffuse nutrient source. According to Bowes et al. (2008), nutrients introduced by point sources tend to decrease in concentration downstream as the consistent input becomes diluted, whilst concentrations from diffuse inputs increase downstream with river flow. Evidence suggests that the primary cause of declining freshwater quality has shifted from point to diffuse sources from the 20th to the 21st century (Neal et al., 2000b). However, Le Moal et al. (2019) suggest current nutrient pollution is the cumulative result of both STW and agricultural inputs.

STWs are recognised as a common point source of nutrient pollution in freshwater bodies. For example, at the River Kennet in North Wessex, Palmer-Felgate et al. (2008) correlated hourly and diurnal fluctuations in concentrations of total P to untreated stormwater effluent discharges from STW, with average values exceeding the standard $100\mu gPL^{-1}$ threshold by $20\text{-}50\mu gPL^{-1}$. However, the influence of this source has reduced markedly since tertiary treatment became mandatory at large STW under The Urban Wastewater Treatment Regulations (1994). For example, biofilter reed beds have reduced concentrations of soluble reactive P in effluent by 75% at a separate STW in the River Kennet (Neal et al., 2000b). According to Bowes et al. (2011), tertiary treatment processes also improved the overall water quality of the River Frome, Somerset from 2002-2009.

However, recent research suggests that improvements to effluent treatment requirements have increased the influence of nutrients from point and diffuse agricultural sources (Bowes et al., 2015). Diffuse sources include the intermittent leaching of fertiliser and faeces from livestock with rainfall. According to Bowes et al. (2005), these sources typically introduce N as opposed to P. For example, a 13-year study by MacDonald et al. (1995) found maximum concentrations of nitrate and ammoniacal nitrogen coincided with riparian arable fertiliser application and cropping at the River Ythan, Scotland. However, Bowes et al. (2015) have also correlated increases in total P ($2\mu gL^{-1}$) with heavy rainfall events downstream of arable farms at the River Frome. Conversely, Bond et al. (2014) found that faeces from livestock had a limited influence on N and P loading in the River Meon, Droxford, with a minor downstream increase of $0.0036mgL^{-1}$ and $0.002mgL^{-1}$, respectively.

Agricultural point inputs include the abstraction of freshwater for crop rinsing and bed cleaning at salad and watercress farms, with the resulting effluent discharged after filtration (Wade et al., 2004). These effluents largely consist of nutrients, pesticides and gravel. Watercress farms occupy a large proportion of chalk stream catchments due to consistent aqueous temperatures and clean, alkaline springs that provide optimal conditions for cultivation (Casey et al., 1993). Consequently, previous studies have identified elevated monthly concentrations of PO_4^{3-} and ammoniacal nitrogen downstream of watercress farms along chalk streams in Dorset and Hampshire, including the River Itchen (Casey, 1981; Casey & Smith, 1994). However, the EA established a $0.045mg/L^{-1}$ limit on reactive P within watercress effluent discharges to the Itchen in 2016.

2.3.1. Fish Farms

Fish farming involves rearing cultivated fish and molluscs for commercial purposes (Sather et al., 2006). Globally, fish farming has increased by 6.9%-per-year since 1976, with a large proportion of freshwater farms occupying chalk streams in southern England (FAO, 2022). However, most studies of nutrients and water quality in UK chalk streams have largely neglected the influence of this source in favour of more common STW and agricultural inputs (Neal et al., 2010). Nevertheless, previous research across Europe suggests the recent rise in freshwater aquaculture has stimulated a consequential surge in nutrient outputs from effluent, faeces and food waste in freshwater systems.

2.3.1.1. Effluent

Effluent can be a critical primary source of nutrients from freshwater fish farms. However, European legislation concerning the composition and frequency of discharges is still largely based on outdated guidelines, as discussed by Rosenthal (1994). A study based in Quebec, Canada suggests that effluent from freshwater fish farms contains 20-25-times less nutrients than effluent from STW (Naylor et al., 2003). However, Foy (2007) suggests fish farm effluent is discharged continuously over a high flow rate, resulting in extreme P loading into freshwater systems with limited seasonal variation.

According to Bergheim & Brinker (2003), nutrient loading from fish farm effluent has reduced by 50-70% in Europe due to improved effluent treatment processes. Previously, rapid solids removal was the dominant effluent treatment technique, where coarse faecal matter is separated from effluent within a sheath (Mayer & McLean, 1995). However, this was vulnerable to shear current forces. Consequently, most farms now use microscreening to remove coarse and fine particles (Pajooh et al., 2016). Nevertheless, significant nutrient loading events can occur due to human and technical errors in treatment processes, as identified in a recent study using macroinvertebrate biomarkers in the Chehel Chai River, Turkey (Gholizadeh & Zibaei, 2020).

2.3.1.2. Faeces

According to Talbot et al. (1999), improvements to effluent treatment have increased the significance of faeces as a nutrient input from freshwater fish farms. Most farms use a feed supplemented with a standardised concentration of PO_4^{3-} to maximise growth rates and economic profits, as opposed to meeting species requirements (Ang & Petrell, 1998). This can lead to the excretion of excess particulate PO_4^{3-} within faeces to deplete the water quality of rivers (Ouaissa et al., 2018). For example, a study in Northern Ireland correlated 25.6kg of annual PO_4^{3-} loadings to faeces from a nearby trout farm. However, fish retained more P from feed (2.2%) as temperatures declined toward Winter.

Consequently, cultivated fish diets can be modified to minimise PO_4^{3-} concentrations within faecal matter. For example, Berzi-Nagy et al. (2021) found fishmeal to exhibit limited digestibility to stunt growth rates and limit nutrient retention in cultivated fish. In comparison, low-fat, high-protein plant meal diets can provide comparable net yields without additional nutrient loading (White et al., 2013). The success of dietary management to control nutrient pollution is exemplified by Clear Spring Foods, who reduced total P in feed by 0.4% to reduce nutrient loading by 10kg-per-tonne-of-fish, with no change to growth rates (MacMillan et al., 2003). However, whilst feed type can influence growth rates and nutrient retention, Berzi-Nagy et al. (2021) found diet to exert a limited impact on the surrounding water quality. Therefore, additional research is needed to assess the influence of nutrient loading from cultivated fish faeces on water quality parameters.

2.3.1.3. Food Waste

Freshwater fish farms can also contribute to nutrient loading through pellet waste. Traditionally, fish farms have used feed management tables to progressively increase the quantity and frequency of feed delivered to fish to maximise growth rates (White et al., 2013). However, this strategy fails to consider diurnal, seasonal and annual fluctuations in feeding rhythms, resulting in food waste and PO_4^{3-} loading (Neal et al., 2005; Noble et al., 2007). For example, a study by Ang & Petrell (1998) found diurnal nutrient concentrations to increase downstream of a freshwater fish farm due to constant excess feed outputs, with waste pellets exceeding the detection range of cultivated salmonids. Furthermore, Pajooh et al. (2016) correlated a downstream increase in concentrations of reactive P ($0.02mgL^{-1}$) to an increase in total suspended solids (TSS) ($27.5mgL^{-1}$) from uneaten pellets at a freshwater fish farm in the Gamasab River, Iran. Food waste can also clog the gills of brown trout and disrupt foraging and migration patterns (Berli et al., 2014).

However, studies have found that wild fish can intercept and consume 90% of waste feed from fish farms to buffer nutrient loading, suggesting this nutrient source is relatively minimal in comparison to effluent and food waste (Håkanson et al., 1998). Furthermore, emerging auto-feed systems can be used to deliver discrete meals timed to coincide with maximum appetite to minimise food waste (Ouissa et al., 2018). Additional mitigation technologies include ultrasound and underwater video to directly observe subsurface feeding and waste pellets (Derrow et al., 1996).

2.4. Sediment Adsorption of Nutrients

Research suggests that within-stream processes can buffer nutrient loading and improve overall water quality downstream of pollutant sources. An example of this is sediment adsorption, where dissolved inorganic PO_4^{3-} can bind to iron and calcium hydroxides at the solid-liquid interface of sediment to lower aqueous concentrations (Gomez et al., 1999). In general, soluble PO_4^{3-} can readily bind to sediment (Li et al., 2021). However, fine sediments such as chalk can adsorb a greater proportion of PO_4^{3-} due to a higher specific surface area (Meng et al., 2014; Li et al., 2021). Furthermore, organic matter within sediments can act as an electron donor to induce redox and pH changes and accelerate PO_4^{3-} adsorption (Gomez et al., 1999). For example, Fadaeifard et al. (2012) hypothesised that adsorption to fine, organic sediment reduced nutrient concentrations 1500-metres downstream of freshwater fish farms in the Karun River, Iran.

However, heavy rainfall and turbid currents can resuspend sediment to reintroduce PO_4^{3-} to the freshwater environment. For example, high precipitation events have been correlated to the remobilisation of sediment and elevated aqueous nutrient levels along the River Swale in Yorkshire (Bowes & House, 2001). Furthermore, Jarvie et al. (2012) found that remobilised PO_4^{3-} previously introduced by a STW could account for one-third of total P loads in the Illinois River, USA, even after effluent treatment processes. Therefore, it is critical to consider the influence of within-stream adsorption and resuspension on nutrient concentrations to holistically assess the temporal and spatial influence of pollution sources in water quality studies (Li et al., 2021).

2.5. Effects of Nutrients on Water Quality Parameters

Excess concentrations of PO_4^{3-} can stimulate algal growth and initiate the cascading process of eutrophication, as demonstrated by the flowchart in Figure 3 (Clark et al., 2017). As the primary production and density of algae increase, chalk stream temperatures and turbidity rise to reduce the photosynthetic production of oxygen, which ultimately acidifies the alkaline pH of chalk streams to create hypoxic conditions and stress in flora and fauna, as seen in Figure 3 (Kang et al., 2018). The remaining oxygen is then consumed by the bacterial decomposition of subsequent dead organic matter (Jarvie et al., 2006).

Consequently, eutrophication can have significant implications for the stable physiochemical parameters of chalk streams (O'Neill, 1998, p.135; Mallin & Cahoon, 2020). Studies of the Rivers Kennet and Swale in the UK have observed small changes in pH and temperature downstream of STW (Neal et al., 2000a; Bowes et al., 2011). However, more significant variations have been identified downstream of fish farms. For example, particulate food and faecal waste have been found to raise aerobic microbial decomposition rates to lower aqueous concentrations of dissolved oxygen by 5mgL-1, and raise electrical conductivity by $2mgL^{-1}$ across rivers in Portugal, Argentina and Poland (Boaventura et al., 1997; Gabellone et al., 2005; Debska et al., 2021).

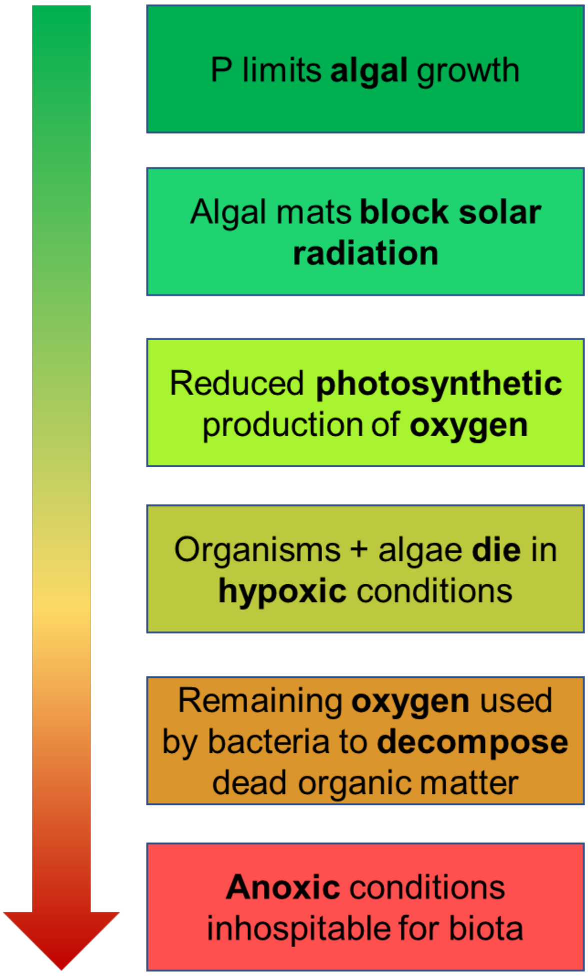


Figure 3. Flowchart of eutrophication in freshwater systems. Information from Clark et al. (2017).

2.6. Effects of Nutrients on Chalk Stream Biota

The stable physiochemical parameters of chalk streams support a range of salmonid, invertebrate and flora communities. Although the impacts of nutrient pollution on ecology are largely uncertain, many species are sensitive to any change in dissolved oxygen, alkalinity or temperature as a consequence of nutrient pollution and eutrophication (Fones et al., 2020).

For example, Lappalainen (2002) found that salmonid spawning is hindered by anoxic, acidic conditions stimulated by eutrophication. Furthermore, Ortiz & Puig (2007) and Friberg et al. (2010) found a 20% decline in invertebrate taxa due to physiochemical disruption downstream of a point nutrient source, with stoneflies, mayflies and caddisflies only present upstream. Durance & Ormerod (2009) also identified shifts in macroinvertebrate assemblages in response to acidifying pH in 50 UK chalk streams. At the River Itchen specifically, Salmon & Trout Conservation (2018) also identified a reduction in 4 mayfly species from 2016-2018, relative to 1978-1982 due to nutrient stress. According to Larsen et al. (2011), larvae of the blue-winged olive (*Serratella ignita*) are also particularly sensitive to elevated P concentrations.

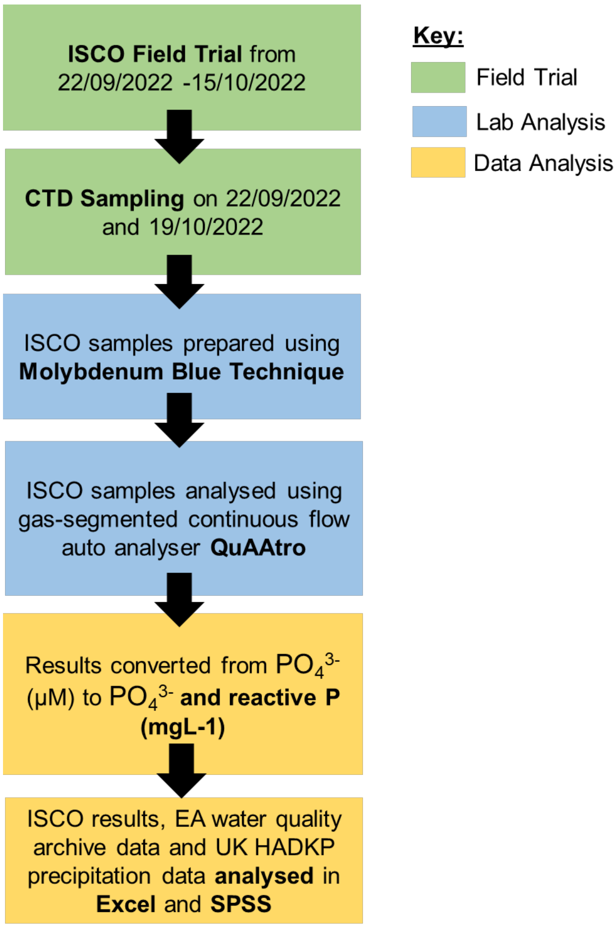
PO_4^{3-} enrichment and eutrophication can also disrupt the competitive balance between algae and aquatic plants. For example, a study of two UK rivers by Carr & Gouder (1990) found nutrients introduced by freshwater fish farms led to extensive algal growth to lower water turbidity for macrophytes further downstream. Thick algal mats have also been consistently observed at the Itchen alongside elevated PO_4^{3-} concentrations from 2016-2018 (Salmon & Trout Conservation, 2018). A range of botanical species in chalk streams provide food, shelter and breeding habitats for salmonids, semi-aquatic birds and riparian mammals, including the protected water vole (Mainstone & Parr, 2002). Therefore, any change in botanical diversity and abundance could impact a wider range of species. More extensive research is needed to understand the ecological implications of point and diffuse nutrient loading in chalk streams, particularly in relation to fish farms.

2.7. Summary

Although recent government reports claim water quality in UK freshwater bodies has improved significantly over the past two decades, recent studies suggest a large proportion of rivers and chalk streams remain below ‘good’ UKTAG WFD status. Previous studies have extensively covered the influence of pollutants from STW and agriculture on the water quality of UK chalk streams. However, the influence of PO_4^{3-} from freshwater fish farms has been largely neglected. Research suggests that improvements to effluent treatment processes at fish farms have increased the significance of waste feed and cultivated fish faeces as nutrient sources in freshwater bodies. However, within-stream sediment adsorption and the behaviour of wild fish can also serve to buffer any nutrient loading over long distances. Studies suggest the exceptional standard of water quality in chalk streams is highly sensitive to nutrient pollution, with excess PO_4^{3-} capable of disrupting stable physiochemical parameters and initiating stress in rare macrophyte, invertebrate and salmonid species. In conclusion, this study is needed to understand the influence of nutrient pollution from freshwater fish farms on water quality parameters at the River Itchen, Hampshire to inform future mitigation and management strategies. Consequently, potential disruption to stable physiochemical parameters and ecological communities can be minimised.

3. Methodology.

This section outlines the field, laboratory and statistical analysis approach for this study, as summarised in Figure 4. Figure 5 shows the location of field sampling sites across the study area.



ISCO Field Trial from 22/09/2022 -15/10/2022

CTD Sampling on 22/09/2022 and 19/10/2022

ISCO samples prepared using **Molybdenum Blue Technique**

ISCO samples analysed using gas-segmented continuous flow auto analyser **QuAAtro**

Results converted from PO_4^{3-} (μM) to PO_4^{3-} **and reactive P (mgL-1)**

ISCO results, EA water quality archive data and UK HADKP precipitation data **analysed** in **Excel** and **SPSS**

Figure 4. Flowchart of the methodology for the study.

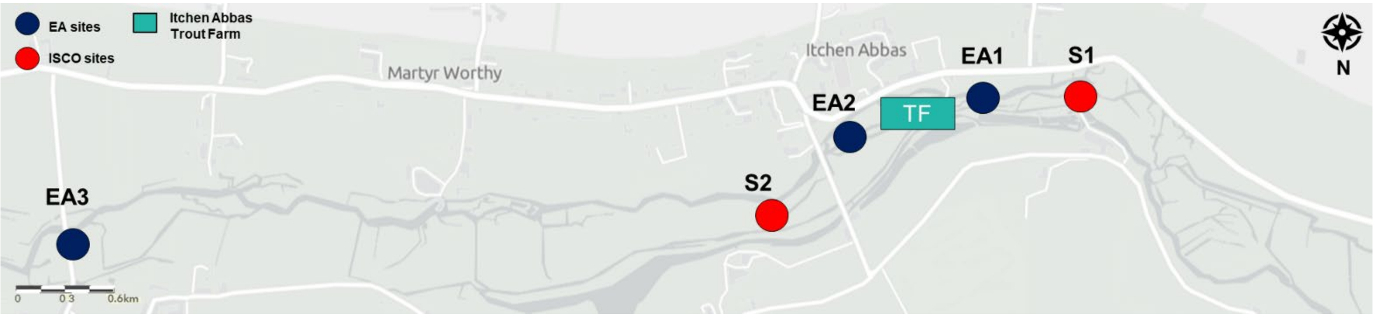


Figure 5. Map of the ISCO sampling sites (S1-2), EA sampling sites (EA1-3) and Itchen Abbas Trout Farm at Itchen Abbas. From Ordinance Survey (2022).

3.1. Field Trial

As seen in Figure 5, two ISCO automated bottle samplers (ABS) were deployed at one site upstream (S1) and one site downstream (S2) of Itchen Abbas Trout Farm to collect daily river water samples from 22/09/2022-15/10/2022. Photographs of the study site and equipment are shown in Figure 6. Samples were collected once every 24-hours over 24-days at 10:16am at S1, and 11:28am at S2. A two-rinse cycle was programmed to limit contamination and ensure samples were representative (Jarvie et al., 2006). Samples were then transferred and stored in the laboratory. This method was selected for this study as it provides insight into high-frequency, diurnal variations in nutrient concentrations with minimal financial costs and time spent at sampling sites, as opposed to traditional manual spot-sample techniques (Knutsson et al., 2013). Alternative approaches include passive diffusive gradients and ChemCatcher technology to obtain weekly time-weighted average concentrations (Fones et al., 2020).

A CTD probe was also used to measure chemical parameters at S1-2 on 22/09/2022 (10am) and 19/10/2022 (12pm), as seen in Figure 6. These parameters included temperature (°C), conductivity (mgL^{-1}) and dissolved oxygen (% and mgL^{-1}), as advised by the Environment Act (2021). Due to a technical fault with the CTD probe, pH could not be measured on either sampling date.

S1

S2

ISCO ABS

ISCO probe in-situ at S1

CTD probe in-situ at S2

Figure 6. Field photographs of ISCO and CTD sampling at S1-2.

3.2. Laboratory Analysis

All river water samples were analysed within a month after their collection at the accredited (ISO 9001:2015) University of Portsmouth Laboratory. 50mL samples were filtered (<0.70µm), and stored at 4°C until analysis. Samples were prepared using the molybdenum blue colourimetry method and the Beer-Lambert Law, as described by Murphy & Riley (1962), Swineheart (1962) and Nagul et al. (2015). This standardised technique is considered the most accurate and time-efficient method for analysing concentrations of P in freshwater (Neal et al., 2000b; Shaw et al., 2021). Alternative strategies include inductively coupled plasma, mass spectrometric or atomic emission detection (Fones et al., 2020).

River water samples were then analysed for concentrations of PO_4^{3-} using the gas-segmented continuous flow auto-analyser QuAAtro. The instrument was calibrated daily using serial dilution. Blanks were included to eliminate noise and identify the limit of detection as 0.02µm (3-times the standard deviation of 12 measurements).

3.3. Unit Conversions

Laboratory analysis provided PO_4^{3-} concentrations for S1-2 in micromoles-per-litre (µM). PO_4^{3-} concentrations were converted into micrograms-per-litre (mgL^{-1}) by multiplying by the molecular mass of PO_4^{3-} (95-grams/mole) and dividing by 1000. Concentrations of PO_4^{3-} (mgL^{-1}) were then multiplied by the molecular mass of oxygen (0.326-grams/mole) to provide concentrations of reactive P (mgL^{-1}) to compare to UK WFD thresholds and EA data.

3.4. Environment Agency Archive

The EA (2023) for southwest England collects monthly spot samples of river water along the Itchen for the statutory monitoring of water quality. For this study, three EA sampling sites (EA1-3) were selected to analyse spatial and temporal trends in water quality parameters from 2000-2022, as seen in **Figure 5.** EA sites did not precisely coincide with ISCO sites due to logistical reasons; therefore, sites in the closest proximity were compared. EA3 was included to analyse variations in nutrients and water quality further downstream of the trout farm (1500-metres).

Over time, the chemical parameters sampled at each EA site have varied significantly. Therefore, no consistent data for conductivity, dissolved oxygen (mgL^{-1}) or temperature was available for comparison with CTD data. However, dissolved oxygen (%), ammoniacal nitrogen as N (mgL^{-1}) and orthophosphate as reactive P (mgL^{-1}) have been consistently monitored at each site and were analysed in this study.

3.5. UKTAG WFD

The UKTAG WFD (2013) define water quality thresholds for ‘high’, ‘good’, ‘moderate’ and ‘poor’ ecological status based on concentrations of reactive P (mgL^{-1}) in freshwater bodies, as seen in Appendix A. These guidelines consider the altitude and alkalinity of rivers. Concentrations of reactive P from the ISCO ABS and the EA water quality archive were compared to these thresholds to infer the status of water quality at each site.

3.6. HadUKP Archive

Daily precipitation (mm) for South East England was obtained from the Met Office (2023) HadUKP for the field trial period (22/09/2022 to 15/10/2022). This data was compared to ISCO concentrations of reactive P to infer whether rainfall had leached nutrients from diffuse sources further upstream to influence water quality at the study area over the trial period. Average annual data from 2000-2022 was also compared to concentrations of nutrients and water quality parameters from the EA archive.

3.7. Statistical Analysis

Statistical analysis was performed using SPSS v.27. A Kolmogorov Smirnov Normality Test was conducted to identify if ISCO, EA and HadUKP data was normally distributed. Descriptive statistics were used to gain an overview of each dataset. Single-Sample-T-Tests were conducted to identify any significant difference in concentrations of reactive P and PO_4^{3-} concentrations between S1-2, and reactive P between EA1-2. A One-Way-ANOVA-Test was also conducted to identify any significant difference in concentrations of reactive P between EA1-3. A Pearson's Correlation Test was used to identify any significant correlations between concentrations of PO_4^{3-} and reactive P (mgL^{-1}) and UK HADKP rainfall data from 22/09/2022-16/10/2022. Finally, a Spearman's Rank was conducted between annual concentrations of EA water quality parameters and UK HADKP rainfall data from 2000-2022 to identify any significant relationships.

4. Results

4.1. Primary Data Collection

4.1.1. ISCO Phosphate

Diurnal concentrations of ISCO-sampled PO_4^{3-} and reactive P were revealed as normally distributed by the Kolmogorov-Smirnov normality test (Appendix B, Table A). Table 1 shows the descriptive statistics for ISCO-sampled concentrations of PO_4^{3-} and reactive P. S2 exhibited greater minimum, maximum and mean average concentrations than S1. S2 also experienced a greater dispersal about the mean, with a larger standard deviation, variance and overall range (Table 1).

Table 1. Descriptive statistics for ISCO-sampled PO_4^{3-} and reactive P at S1-2.

	Minimum	**Maximum**	**Mean Average**	**Std. Deviation**	**Variance**
S1 reactive P (mgL^{-1})	0.006	0.055	0.020	0.132	0.000
S2 reactive P (mgL^{-1})	0.020	0.970	0.583	0.224	0.001
S1 PO_4^{3-} (mgL^{-1})	0.017	0.169	0.619	0.406	0.002
S2 PO_4^{3-} (mgL^{-1})	0.061	0.296	0.179	0.687	0.005

Figure 7 shows the variation in concentrations at S1-2 over the 24-day trial period. No values breached the limit of detection. Overall, concentrations increased downstream from an average of $0.62mgL^{-1}$ (PO_4^{3-}) and $0.02mgL^{-1}$ (reactive P) at S1, to $0.179mgL^{-1}$ (PO_4^{3-}) and $0.058mgL^{-1}$ (reactive P) at S2. This difference was confirmed as statistically significant by the Paired-Sample-T-Test, with all p-values less than 0.01 (Appendix B, Table B). However, concentrations were similar between S1-2 at ABS 8, with a $0.04mgL^{-1}$ difference in PO_4^{3-} and $0.001mgL^{-1}$ in reactive P. Concentrations also increased temporally from ABS 1-24 in Figure 7, with a greater positive correlation at S1 ($R^2 = 0.7084$). Both sites also experienced numerous spikes in P over several days, which were more frequent and of a larger magnitude at S2.

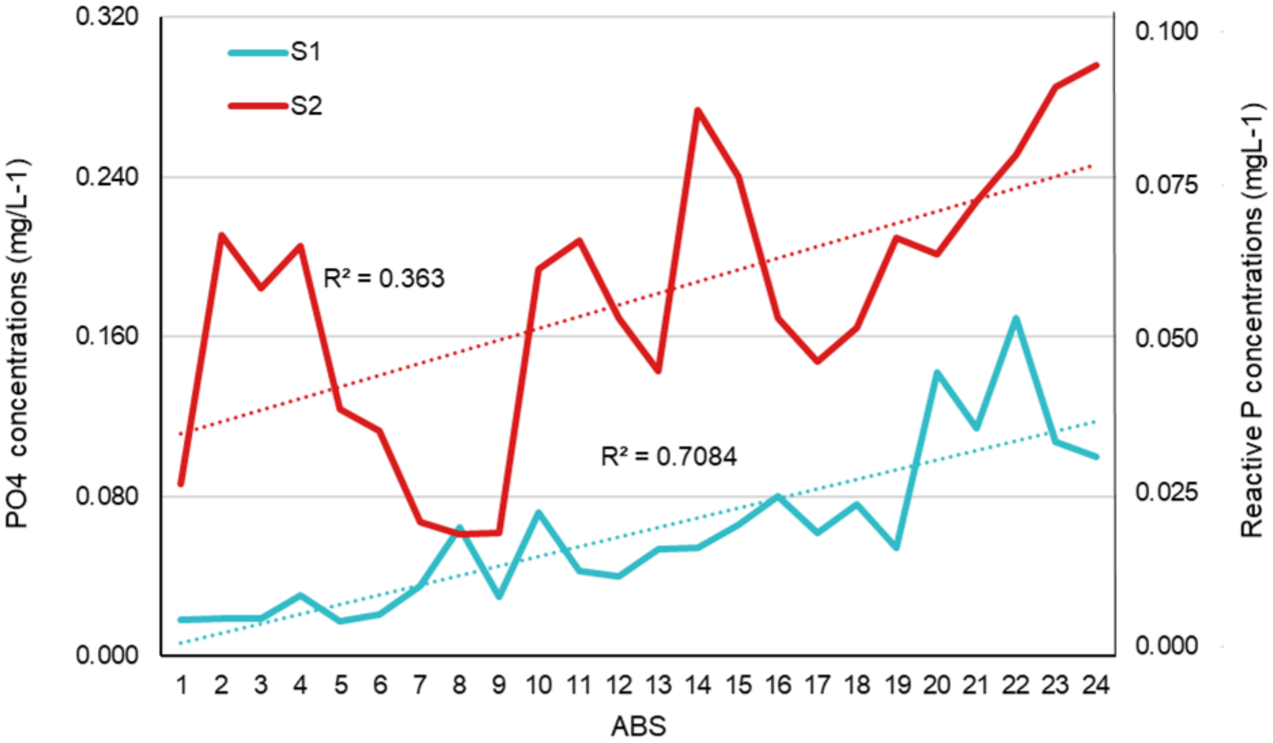


Figure 7. Graph showing concentrations of PO_4^{3-} and reactive P from ABS 1-24 at S1-2.

4.1.2. CTD Water Quality Parameters

Table 2 shows the results of CTD spot sampling over the 24-day trial period. Temperature and conductivity increased downstream by an average of 0.23°C and 6.05µscm^{-1} respectively. Concentrations also increased temporally from 22/09/2022-19/10/2022. Conversely, dissolved oxygen (% and mgL^{-1}) reduced downstream for both sample dates, by an average of 31.15% and 3.5mgL^{-1} respectively.

Table 2. Results of the CTD-probe spot analysis at S1-2 over the 24-day trial period.

	22/09/2022		**19/10/2022**		**Average**	
	S1	**S2**	**S1**	**S2**	**S1**	**S2**
Temperature (°C)	10.70	11.05	11.30	11.41	11.00	11.23
Conductivity (µs/cm)	420.70	427.20	427.50	433.10	424.10	430.15
Dissolved O2 (%)	103.20	77.10	102.90	66.70	103.05	71.9
Dissolved oxygen (mgL^{-1})	11.47	8.47	11.26	7.27	11.37	7.87

4.2. Comparison with UKTAG WFD Thresholds

Concentrations of reactive P sampled by the ISCO ABS were compared to UKTAG WFD thresholds in Figure 8 to determine the water quality status of the Itchen Abbas catchment. As seen in Figure 8, water quality degraded from ‘high’ to ‘good’ status from S1-2. Concentrations from ABS 14-15 and 21-24 breached the average threshold for ‘good’. Only ABS 24 exceeded the upper limit of 0.91mgL^{-1} to reach ‘moderate’ status (Appendix A).

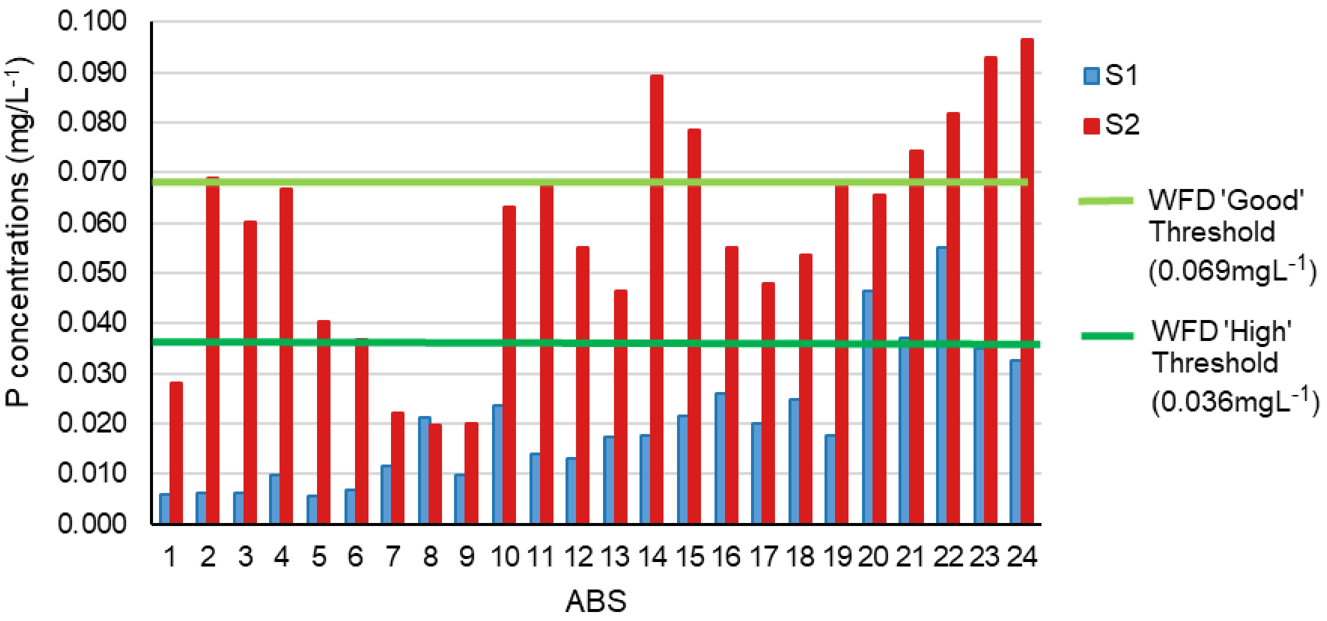


Figure 8. Graph comparing ISCO reactive P concentrations at ABS 1-24 to UKTAG WFD thresholds. Data from UKTAG WFD (2013).

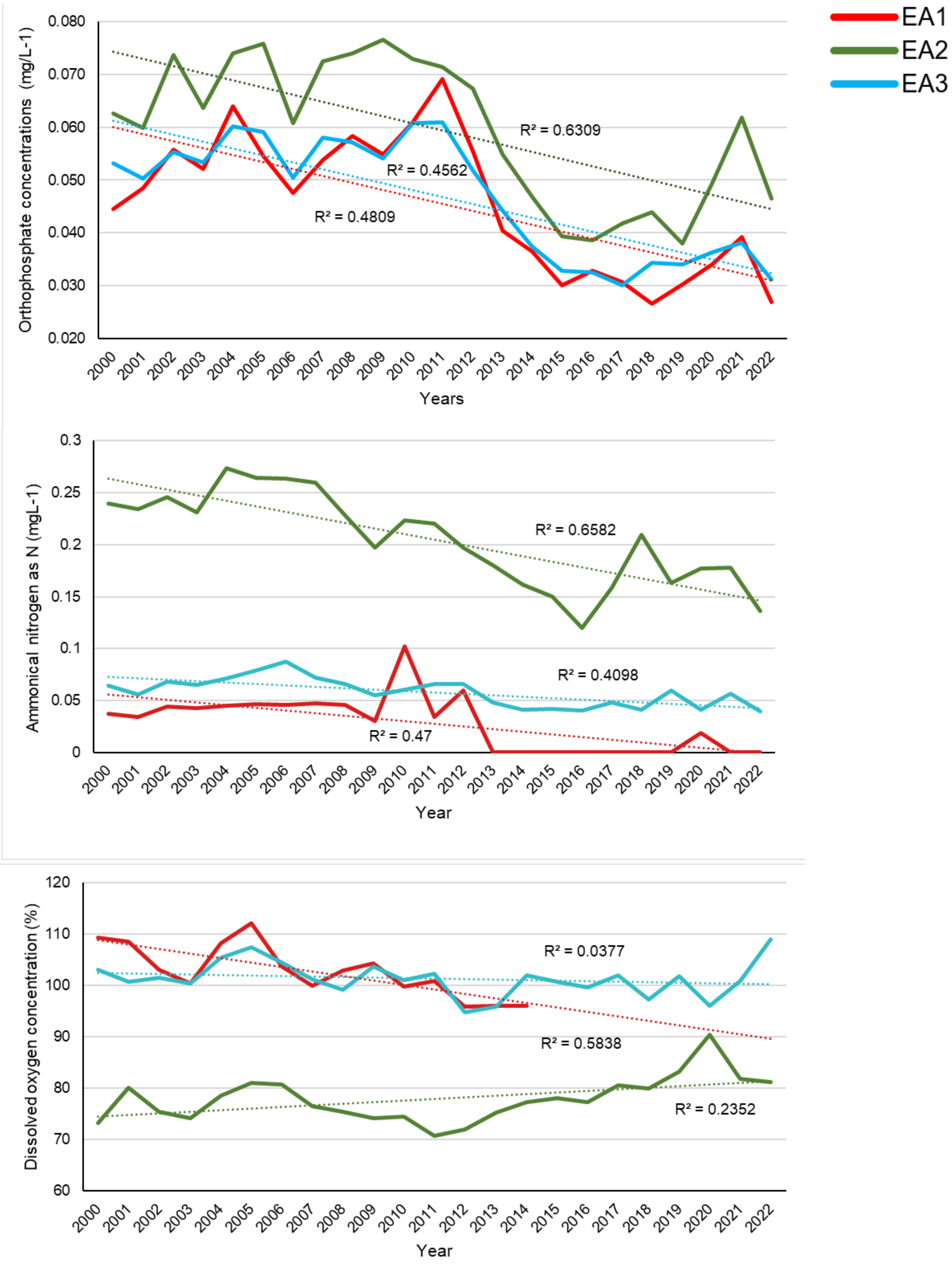
4.3. Trends in EA Archive Water Quality Data

The Kolmogorov-Smirnov normality test revealed monthly concentrations of all parameters from the EA archive were not normally distributed (Appendix B, Table C). The descriptive analysis results for monthly and annual concentrations are shown in Table 3. Standard deviation and variance are proportional to the magnitude of the base data. Therefore, it was of little value to compare these between parameters, as the magnitude of base data varied significantly.

Table 3. Descriptive statistics for monthly and annual EA spot-sampled P at EA1-3.

		Minimum	**Maximum**	**Mean**	**Std. Deviation**	**Variance**
Annual	**Orthophosphate as P (mgL^{-1})**	0.027	0.077	0.051	0.014	0.000
	Dissolved O2 (%)	70.760	112.160	92.816	12.430	154.517
	Ammoniacal Nitrogen as N (mgL^{-1})	0.030	0.274	0.101	0.080	0.006
Monthly	**Orthophosphate as P (mgL^{-1})**	0.012	0.330	0.052	0.026	0.001
	Dissolved O2 (%)	56.400	159.900	92.694	16.600	275.574
	Ammoniacal Nitrogen as N (mgL^{-1})	0.019	0.448	0.103	0.090	0.008

In general, average concentrations of orthophosphate and ammoniacal nitrogen increased downstream from EA1-2, whilst dissolved oxygen reduced. These trends typically recovered by EA3. Spatial differences were confirmed as statistically significant by the Paired-Sample-T-Test between EA1-2, and the One-Way-ANOVA-test between EA1-3, with all p-values less than 0.01 (Appendix B, Tables D-E). Annual trends in concentrations of orthophosphate, ammoniacal nitrogen and dissolved oxygen at EA1-3 from 2000-2022 have been graphed in Figures 9-11, with each parameter discussed in more detail below.



Figures 9, 10 and 11. Annual concentrations of orthophosphate, ammonia and dissolved oxygen spot sampled at EA1-3 from 2000-2022. Data from EA (2023).

4.3.1. Orthophosphate

Overall, concentrations of orthophosphate for September 2022 were marginally less than average concentrations of reactive P measured by the ISCO ABS over the 24-day trial period, by 0.006mgL^{-1} upstream and 0.002mgL^{-1} downstream. Data for October 2022 was unavailable for comparisons due to the inconsistent nature of EA spot sampling.

From 2000-2022, average monthly concentrations of orthophosphate increased from 0.046mgL^{-1} at EA1 to 0.06mgL^{-1} at EA2, before declining toward 0.048mgL^{-1} at EA3. However, significant monthly variation occurred during the study period, with an overall range of 0.318mgL^{-1} (Table 3). Furthermore, 12 anomalous increases in monthly concentrations occurred from EA2-3, out of 242 total samples (December 2007, June 2013, September 2016, November 2016, June 2019). Although most concentrations fell within the ‘high’ and ‘good’ UKTAG ecological thresholds, some measurements at EA2 breached the range of ‘moderate’ (October 2004, September 2005, September 2009, July 2011), as seen in Appendix A.

A tentative seasonal trend was also identified in orthophosphate, with concentrations typically peaking in Autumn at 0.08mgL^{-1} (EA1 and EA3) and 0.1mgL^{-1} (EA2), before declining toward 0.02mgL^{-1} and 0.04mgL^{-1} respectively in Winter. Conversely, annual concentrations remained largely stagnant from 2000-2009, at ~0.045mgL^{-1} (EA1 and EA3) and ~0.07mgL^{-1} (EA2), as seen in Figure 9. However, a large decline of 0.03mgL^{-1} occurred from 2010-2019 at all sites. This was followed by a marginal increase of 0.01mgL^{-1} (EA1 and EA3) and 0.02mgL^{-1} (EA2) toward 2021 which corrected itself by 2022. Overall, EA2 exhibited the strongest negative correlation, with a R^2 value of 0.6309 (Figure 9).

4.3.2. Ammoniacal Nitrogen

Average monthly concentrations of ammoniacal nitrogen followed a similar spatial trend to orthophosphate, increasing downstream from an average of 0.043mgL^{-1} (EA1) to 0.208mgL^{-1} (EA2), before declining toward 0.061mgL^{-1} (EA3). However, concentrations at EA3 typically exceeded those at EA1. Furthermore, this parameter experienced a significant range in monthly concentrations across all three sites, at 0.429mgL^{-1} (Table 3).

Monthly concentrations of ammoniacal nitrogen followed an inverse seasonal trend to orthophosphate. Values peaked in Spring at 0.05-0.1mgL^{-1} (EA1 and EA3) and 0.2-0.3mgL^{-1} (EA2), before declining toward Winter by ~0.1mgL^{-1}. As seen in Figure 10, annual concentrations of ammoniacal nitrogen generally declined from 2000-2022 by ~0.1mgL^{-1} (EA2) and ~0.05mgL^{-1} (EA1 and EA3), with similar R^2 values to orthophosphate (Figure 9). However, short-term increases occurred from 2003-2006 and 2015-2017. There was a significant range in annual concentrations of 0.244mgL^{-1}; however, this was almost half that observed for monthly concentrations (Table 3).

4.3.3. Dissolved Oxygen

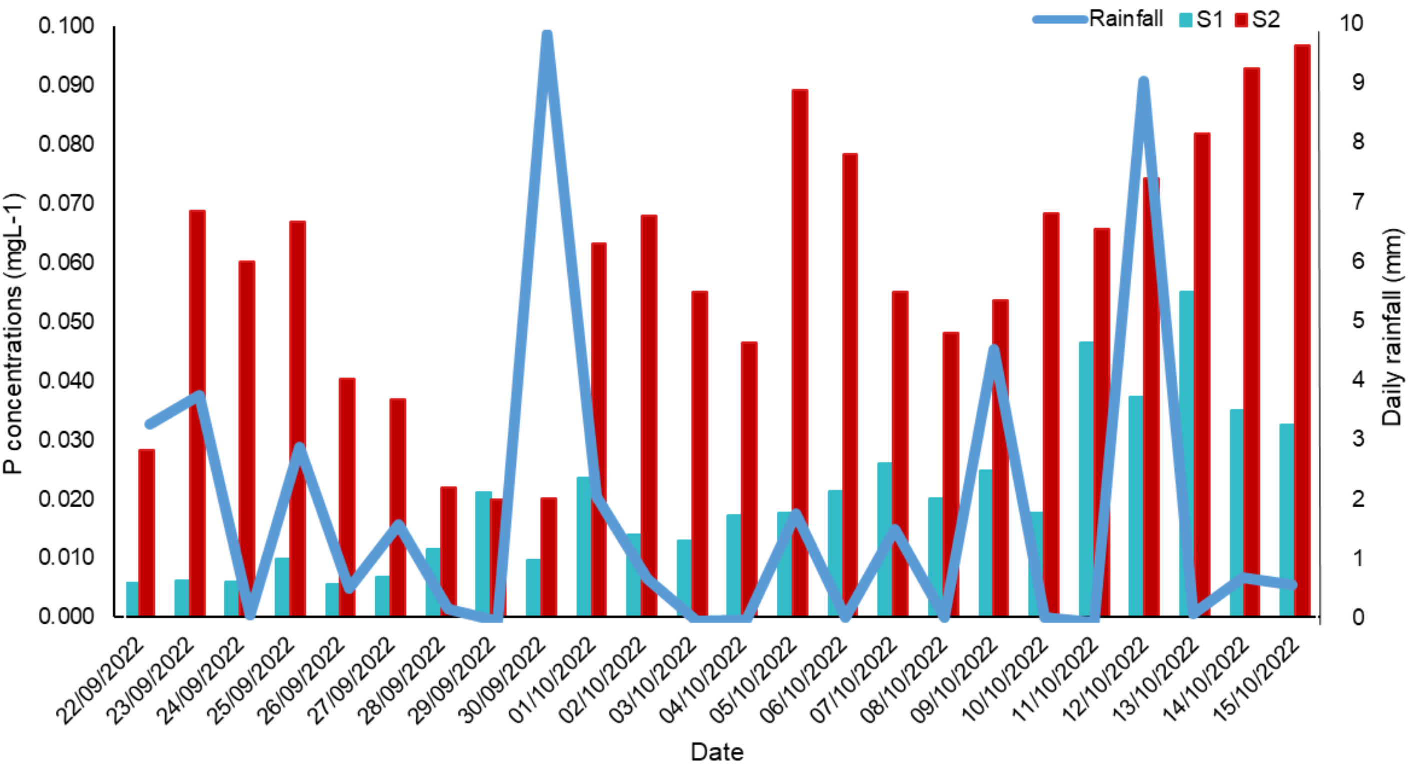
From 2000-2022, dissolved oxygen decreased from a monthly average of 102.958% (EA1) to 77.504% (EA2), before increasing toward 101.656% (EA3). As seen in Table 3, there was a significant variation in annual and monthly concentrations, with a range of 42.6% and 103.4%, respectively.

Monthly concentrations of dissolved oxygen followed a similar seasonal trend to ammoniacal nitrogen, with values peaking in Spring/Summer at 80% (EA2) and 100-120% (EA1 and EA3), before declining toward Winter by 10-20%. As seen in Figure 11, annual trends were relatively consistent, with smaller R^2 values than Figures 9-10. Concentrations at EA1 and EA3 were very similar at ~100% and were consistently 10-20% greater than concentrations at EA2. However, these observations are limited by a lack of data available for EA1 from 2014. Consequently, only measurements at EA2 could be compared to ISCO samples, with a minor difference of 3% between EA2 (September 2022) and S2 (24-day field trial).

4.4. Comparison of HadUKP Rainfall Archive with ISCO and EA data

Daily rainfall from HadUKP over the 24-day field trial was plotted against ISCO concentrations of reactive P in Figure 12. The average rainfall during this period was 1.668mm. However, diurnal measurements varied significantly, ranging from 8.95mm (30/09/2022) to 0mm (11/10/2022) with no general positive or negative trend. Annual rainfall from 2000-2022 was more consistent at ~2mm. This was plotted against annual average concentrations of EA orthophosphate in Figure 13.

Overall, Figures 12-13 demonstrate a lack of correlation between concentrations of reactive P and rainfall. However, several sporadic increases in rainfall over the 24-day field trial were followed by a delayed increase in reactive P, as seen in Figure 12. For example, a 10mm increase in rainfall on 30/09/2022 was followed by a doubling in reactive P at S1-2 from 01/10/2022-02/10/2022. An additional 9mm rise on 12/10/2022 was followed by an increase in reactive P from 0.075-0.1mgL^{-1} at S2 from 13/10/2022-15/10/2022. Nevertheless, the Pearson's Correlation Analysis and Spearman's Rank revealed no significant correlation between rainfall and reactive P over the 24-day trial period and from 2000-2022, with all p-values greater than 0.01 (Appendix B, Table F-G).



Figures 12 and 13*.* Graphs comparing daily precipitation with ISCO reactive P concentrations over the 24-day trial **(Figure 12)**, and annual average precipitation with concentrations of EA orthophosphate from 2000-2022 **(Figure 13).** Data from EA (2023) and Met Office (2023).

5. Discussion

5.1. Variation in Phosphorus

5.1.1. ISCO ABS

Figure 14 summarises the spatial variation in average concentrations of reactive P from ISCO sampling (24-day trial) and the EA archive (September 2022). As seen in Figure 14, average ISCO-sampled concentrations increased downstream of the fish farm from S1-S2, which was confirmed as statistically significant by the Paired-Sample-T-Test. This trend suggests that Itchen Abbas Trout Farm influenced concentrations of reactive P 50-metres downstream over the 24-day field trial. Concentrations sampled at ABS 8 were very close to the detection limit, which explains the anomalous similarity between S1-2.

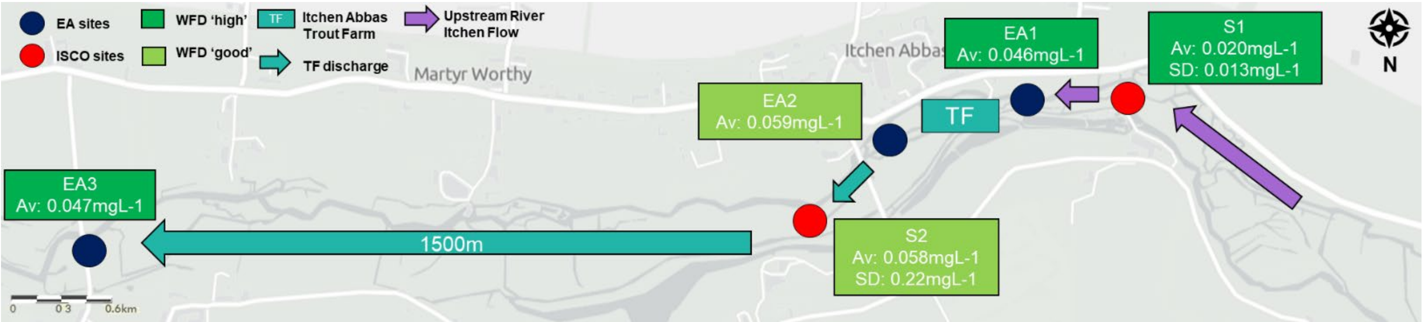


Figure 14. Map of average ISCO and EA September 2022 reactive P concentrations and their UKTAG WFD status across Itchen Abbas, alongside discharge pathways from Itchen Abbas Trout Farm and upstream River Itchen Flow. From OpenStreetMap (2022).

Although several delayed increases in reactive P were observed after spikes in rainfall over the 24-day trial period, these were not confirmed as statistically significant by the Pearson's Correlation Analysis. This suggests that point sources, rather than diffuse sources were responsible for diurnal variations in reactive P at S1-2 (Fones et al., 2020). Previous studies of water quality by Foy & Rossel (1991), Ang & Petrell (1998) and Pajooh et al. (2016) have identified similar downstream increases in diurnal reactive P ($0.02mgL^{-1}$) at numerous fish farms. These were accompanied by an increase in TSS of 27.5mgL^{-1} from faeces and uneaten food pellets. Therefore, it is possible that waste from Itchen Abbas Trout Farm was responsible for spatial variations in reactive P over the 24-day trial period. Effluent was unlikely to be responsible for the consistent downstream increase in reactive P seen in Figure 14 due to recent improvements to treatment processes (Bergheim & Brinker, 2003; Naylor et al., 2003). Future studies should consider analysing concentrations of TSS upstream and downstream of the fish farm to confirm this conclusion.

The downstream increase in average reactive P caused water quality to decline from ‘high’ to ‘good’ UKTAG WFD ecological status from S1-2, as seen in Figure 14 (UKTAG WFD, 2013). Whilst several concentrations breached the average ‘good’ threshold, most remained below the upper limit of 0.091mgL^{-1} (Appendix A). According to Defra (2014, p.17), ‘good’ is an acceptable standard for freshwater bodies. This suggests that Itchen Abbas Trout Farm exerted a relatively small influence on catchment water quality over the 24-day field trial. Nevertheless, Durance & Ormerod (2009) claim that macroinvertebrate communities rely on the stable water quality of chalk streams in southern England, with any disruption capable of altering community compositions and the ecosystem services they provide. Therefore, any factor capable of reducing the water quality of chalk streams below ‘high’ UKTAG WFD status should be considered ecologically significant. Future investigation into the invertebrate assemblages at each site would be required to fully assess the ecological implications of nutrient discharges from Itchen Abbas Trout Farm.

Concentrations of PO_4^{3-} and reactive P also varied temporally. In general, concentrations increased over the 24-day trial period, with a greater positive regression at S1. As this site is upstream from the fish farm, it is possible that outputs from alternate sources influenced nutrient concentrations at this site, such as point effluent discharges from watercress farms, diffuse leaching of agricultural fertiliser or septic tank outputs (Casey & Smith, 1994). S2 experienced significant diurnal variation in PO_4^{3-} and reactive P, with greater variance and standard deviation values than S1 from the descriptive statistical analysis. This suggests that even during this short 24-day study there was a significant amount of variation that was missed by low-frequency EA monthly spot samples, as identified in studies by Foy et al. (2007) and Fones et al. (2020). Noble et al. (2007) suggest the feeding rhythms of cultivated fish vary diurnally; however, these patterns are not considered by the standardised approach of feed management tables. Therefore, extended meal endpoints and consequential food waste from Itchen Abbas Trout Farm could be responsible for the diurnal variation in concentrations of PO_4^{3-} and reactive P at S2.

5.2. EA Orthophosphate Archive

As seen in Figure 14, spot samples of orthophosphate at EA1-2 for September 2022 were relatively similar to the mean average reactive P concentrations sampled at S1-2 over the 24-day trial period. Concentrations of reactive P at the fish farm outlet site (EA2) remained relatively unchanged 50-metres downstream at S2, with a difference of 0.001mgL^{-1}. This highlights the significance of this area for nutrient loading and potential ecological implications.

From 2000-2022, average monthly concentrations of orthophosphate typically increased downstream from EA1-2, degrading water quality from 'high' to 'good' UKTAG WFD status, in a similar trend to S1-2 (Figure 14). However, concentrations generally recovered 1500-metres downstream at EA3 to return water quality to its original 'high' status, as seen in Figure 14. This distribution was confirmed as statistically significant by the Paired-Sample-T-Test between S1-2 and the One-Way-ANOVA-test between EA1-3. A similar trend was correlated to within-stream sediment adsorption by Fadaeifard et al. (2012) in a study of the Karun River, Iran. Therefore, it is possible that some of the orthophosphate introduced by Itchen Abbas Trout Farm may have adsorbed onto the fine mineralogy of chalk stream sediment to lower aqueous concentrations at EA3. However, it is critical to consider the potential for rapid currents and heavy rainfall events to resuspend sediment and reintroduce excessive nutrient concentrations to stimulate ecological impacts further downstream, as identified by Bowes & House (2001) and Jarvie et al. (2012). Alternatively, Håkanson et al. (1998) and Neal et al. (2005) suggest wild fish can consume faeces and particulate food waste downstream of fish farms to buffer nutrient loading. Therefore, this could be an additional factor responsible for the decline in concentrations of orthophosphate from EA2-3.

However, several anomalous increases in monthly concentrations of orthophosphate occurred from EA2-3. In most cases, concentrations at EA2 exceeded $0.07 mgL^{-1}$, which correlates to the UKTAG WFD ‘good’ threshold (Appendix A). This suggests $0.07 mgL^{-1}$ could be an upper limit for the adsorption of orthophosphate to sediment in the Itchen Abbas catchment (Meng et al., 2014). Consequently, this threshold could be used to inform future nutrient outlet management at Itchen Abbas Trout Farm to prevent long-distance P loading.

Furthermore, several measurements of orthophosphate at EA2 breached the UKTAG WFD ‘moderate’ category. This suggests Itchen Abbas Trout Farm has the capacity to significantly degrade the water quality of chalk streams as a consequence of sporadic high nutrient outputs. However, according to studies by Carr & Gouder (1990) and Larsen et al. (2011), the rarity of these events suggests any ecological implications would be minimal. Furthermore, the sporadic nature of these measurements suggests another causative factor could be involved, such as river discharge, water sampling errors or effluent treatment issues. For example, systematic errors in microscreening were correlated to sporadically high nutrient outputs from fish farms in Turkey by Gholizadeh & Zibaei (2020).

From 2000-2022, concentrations of orthophosphate exhibited a tentative seasonal trend, contradictory to studies by Foy (2007). Concentrations typically peaked in Autumn and declined toward Winter. Foy & Rosell (1991) suggest cultivated fish retain more P from feed during Winter as temperatures decline. Therefore, this may have lowered orthophosphate loading from the trout farm to reduce aqueous concentrations in Winter from 2000-2022.

Overall, average annual orthophosphate concentrations declined from 2000-2022, with a significant drop from 2010-2019. Similar trends have been identified in numerous long-term studies of water quality in UK rivers and chalk streams (Hilton et al., 2006; Neal et al., 2010; Bowes et al., 2011; Le Moal et al., 2019). This has largely occurred due to improvements in effluent tertiary treatment processes and legislative requirements for point nutrient sources, including the Urban Wastewater Treatment Regulations (1994) and EU WFD (2000). However, concentrations of orthophosphate increased in 2021 and recovered by 2022. This likely occurred due to Covid-19 lockdowns and EA funding cuts which disrupted water quality sampling during this period (Fones et al., 2020).

5.3. Variation in Water Quality Parameters

Spatial and temporal shifts in reactive P from S1-2 and EA1-3 were accompanied by variations in water quality parameters sampled by the CTD probe over the 24-day field trial, and the EA water quality archive from 2000-2022.

5.3.1. CTD Probe

CTD spot-sampling revealed an increase in temperature and conductivity and a decline in dissolved oxygen from S1-2, alongside an increase in reactive P. This suggests that reactive P introduced by the fish farm may have influenced water quality parameters over the 24-day trial period, as previously identified by Boaventura et al. (1997), Gabellone et al. (2005) and Debska et al. (2021) as a consequence of food waste. This contradicts Berzi-Nagy et al. (2021), who claimed that feed type has limited influence on surrounding water quality. Several algal patches were also observed on the water surface of S2, in correlation to previous research at the Itchen (Salmon & Trout Conservation, 2018). Therefore, this suggests concentrations of reactive P from the fish farm were sufficient to stimulate algal growth and raise temperatures and lower water turbidity, photosynthesis and concentrations of dissolved oxygen, as identified in a study of two UK rivers by Carr & Gouder (1990).

Water quality parameters also varied temporally between sampling dates alongside reactive P. However, this could reflect shifts in sampling times. For example, the temperature measured on 19/10/2022 was greater as the sampling time coincided with midday when warming typically peaks. Furthermore, Noble et al. (2007) suggest feeding behaviour in cultivated fish peaks toward midday when maximum light is available to detect pellets and avoid predators. Consequently, cultivated fish are typically fed around 12pm. Therefore, it is possible concentrations of particulate and dissolved food waste may have been greater on 19/10/2022, explaining the temporal increase in conductivity and decline in dissolved oxygen.

5.3.2. EA Archive

The spatial distribution of nutrient concentrations and water quality parameters from the EA archive (2000-2022) were similar to those identified over the 24-day field trial. A general increase in orthophosphate from EA1-2 was accompanied by an overall increase in ammoniacal nitrogen and a decline in dissolved oxygen. These changes recovered further downstream at EA3 alongside concentrations of orthophosphate.

The statistically significant results of the One-Way-ANOVA-test and Paired-Sample-T-Test strongly imply that a causative factor influenced the spatial distribution of parameters from EA1-3. An Iranian study by Fadeifard et al. (2012) suggests that whilst nutrient pollution from freshwater fish farms can disrupt water quality parameters immediately downstream, concentrations can recover at 1500-metres downstream due to the dilution of nutrients from within-stream processes. Consequently, this suggests that Itchen Abbas Trout Farm exerts a limited influence on water quality parameters over distances exceeding 1500-metres due to within-stream processes, such as sediment absorption, contradictory to Berzi-Nagy et al. (2021). However, impacts to water quality parameters below this distance could still be ecologically significant (Ortiz & Puig; Friberg et al., 2010). Furthermore, the recovery in dissolved oxygen at EA3 could simply reflect high river flows, which entrain oxygen to elevate concentrations of dissolved oxygen, as identified by Boaventura et al. (1997).

Both ammoniacal nitrogen and dissolved oxygen exhibited a significant range in concentrations across EA1-3. Maximum concentrations typically occurred at S2, which strongly implies these were caused by significant nutrient-loading events from the fish farm, as identified by (Neal et al., 2000b). However, both parameters exhibited a pronounced seasonal trend, with concentrations peaking in late Spring and declining toward Winter at all three sites. These correlate to seasonal trends identified downstream of arable farms at the Rivers Ythan and Frome by MacDonald et al. (1995) and Bowes et al. (2015). Consequently, fertiliser may have leached from upstream watercress or arable farms in New Alresford to cumulatively influence seasonal concentrations of ammoniacal nitrogen at EA1-3 with the fish farm. Based on the findings of Bond et al. (2014), any faecal loading from livestock farms upstream was likely to be minimal, with an insignificant effect on water quality parameters. As discussed in a study of Dorset chalk streams by Casey & Smith (1994), high concentrations of N in late Spring may have reduced dissolved oxygen to explain similar seasonal trends in these parameters. However, no significant correlation was identified between monthly trends in HadUKP rainfall and concentrations of ammoniacal nitrogen or dissolved oxygen, suggesting any influence from upstream agricultural sources was relatively minimal. Nevertheless, daily precipitation data from HadUKP was only available for the South-East of England; therefore, there may have been local variations in rainfall that were not accounted for in this study.

Annual concentrations of ammoniacal nitrogen declined from 2000-2022, in agreement with studies by Le Moal et al. (2019) and Whelan et al. (2022). This trend reflects the success of legislative water quality monitoring and management under the UKTAG WFD (2013) and EU WFD (2017). However, several anomalous increases occurred from 2003-2004 and 2016-2018. As these do not correlate to any significant increases in rainfall, it is possible that point sources, rather than diffuse sources, could be responsible for these events. Conversely, annual concentrations of dissolved oxygen were mostly consistent from 2000-2022, in agreement with Pajooh et al. (2016). This suggests nutrient pollution from Itchen Abbas Trout Farm was not sufficient to significantly influence annual average concentrations of dissolved oxygen. However, these averages do not highlight the observed 35% decline in monthly concentrations between EA1-2, with similar variation correlated to salmonid and invertebrate community disruption in previous studies (Lappalainen, 2002; Salmon Trout Conservation, 2018). Stable trends could also be a consequence of sporadic EA spot sampling, with measurements ceasing at EA1 from 2014 due to government funding cuts.

5.4. Recommendations for Itchen Abbas Trout Farm

Based on evidence and interpretations within Sections 4-5, this study recommends several nutrient mitigation strategies for Itchen Abbas Trout Farm to minimise any impacts to macrophyte, invertebrate and salmonid communities immediately downstream (50-metres). These strategies have been allocated based on literature and case studies of best practice, as data concerning the composition of effluent, feeding practices and fish stocks are not publicly available for the trout farm.

As discussed in Section 2.3., the most common nutrient source from freshwater fish farms include effluent, food waste and faeces. Evidence from this study suggests that sporadic reactive P concentrations breached the “good” and “moderate” UKTAG WFD categories at S2 and EA2 potentially due to errors in effluent treatment processes (Gholizadeh & Zibaei, 2021). Therefore, Itchen Abbas Trout Farm should consider reviewing and updating current treatment systems to avoid future anomalous outputs (Bergheim & Brinker, 2003).

Temporal and seasonal trends in nutrients and water quality parameters correlated to the feeding behaviour of cultivated fish (Noble et al., 2007). Therefore, Itchen Abbas Trout Farm should consider monitoring trout stocks to adapt approaches to feed delivery (Talbot et al., 1999). A low PO_4^3, high protein diet should also be considered to improve the retention of P in cultivated fish, as exemplified by the Clear Spring Foods case study (MacMillan et al., 2003; Berzi-Nagy et al., 2021). Food waste could also be monitored using underwater cameras, electric pellet counters and sonar systems (Ang & Petrell, 1998).

6. Conclusion

6.1. Key Findings

This study has revealed the influence of nutrients from Itchen Abbas Trout Farm on the water quality of the River Itchen. Downstream increases in reactive P over the 24-day field trial and 2000-2022 EA archive appeared to stimulate shifts in water quality parameters, including an increase in conductivity and temperature, and a reduction in dissolved oxygen. Nutrient trends were largely correlated to food waste and faecal matter from the fish farm, with spike events most likely caused by effluent processing errors. This ultimately lowered water quality from ‘high’ to ‘good’ UKTAG WFD status from S1-2 and EA1-2. Whilst Defra (2014, p.17) consider ‘good’ to be an acceptable standard for freshwater quality, recent research suggests that any disruption to physiochemical parameters in chalk streams can be highly detrimental to salmonid and macroinvertebrate behaviour, reproduction and community compositions. This suggests that WFD classifications for reactive P are not high enough for the natural stability and biodiversity of chalk streams, despite the recent consideration of river altitude and alkalinity by the UKTAG WFD (2013).

Reactive P introduced by Itchen Abbas Trout Farm appeared to impact water quality immediately downstream (50-metres), with recovery seen over a further 1500-metres at EA3. This suggests the importance of monitoring nutrients and additional pollutants over short distances to understand potential implications to water quality and ecology. Annual declines in both reactive P and ammoniacal nitrogen from 2000-2022 indicate the success of monitory and regulatory measures under the WFD. However, these averages do not account for significant diurnal variations that were identified during the 24-day field trial.

Overall, nutrient pollution at Itchen Abbas was most likely the cumulative result of the fish farm and agricultural inputs. Whilst seasonal trends in orthophosphate followed the feeding rhythms of cultivated fish, concentrations of ammoniacal nitrogen and dissolved oxygen correlated to the agricultural calendar. However, no significant correlation was found between rainfall and nutrient concentrations, suggesting influence from further upstream was relatively minimal. It is hoped that the future holistic monitoring of all nutrient input sites under the Environment Act (2021) will also provide greater insight into the influence of fish farms on nutrient loading in UK chalk streams and encourage the introduction of future mitigation strategies.

6.2. Further Research

Further research could involve analysing diurnal variations in additional pollutants, including ammoniacal nitrogen, veterinary drugs and antibiotics, to assess and compare the extent of each pollutant and its influence on freshwater quality (Justino et al., 2016). Concentrations of TSS could also be analysed to understand the diurnal significance of food waste and faeces on water quality, and subsequent effects on the feeding and migration of wild brown trout (Berli et al., 2014).

Additional fractions of P should also be analysed to identify the proportion of dissolved inorganic PO_4^{3-} available for sediment adsorption (Meng et al., 2014). Sediment samples should also be analysed for this fraction to assess the influence of adsorption processes on the distribution of aqueous nutrient concentrations over long distances (>1500-metres).

Finally, an additional third downstream ISCO site should be considered to further investigate the long-distance dispersal and adsorption of nutrients over a diurnal frequency. Further research is also needed to understand the implications of nutrient loading from UK fish farms in relation to feed management, effluent discharge and consequential effects on the ecological communities in UK chalk streams (Salmon & Trout Conservation, 2018).

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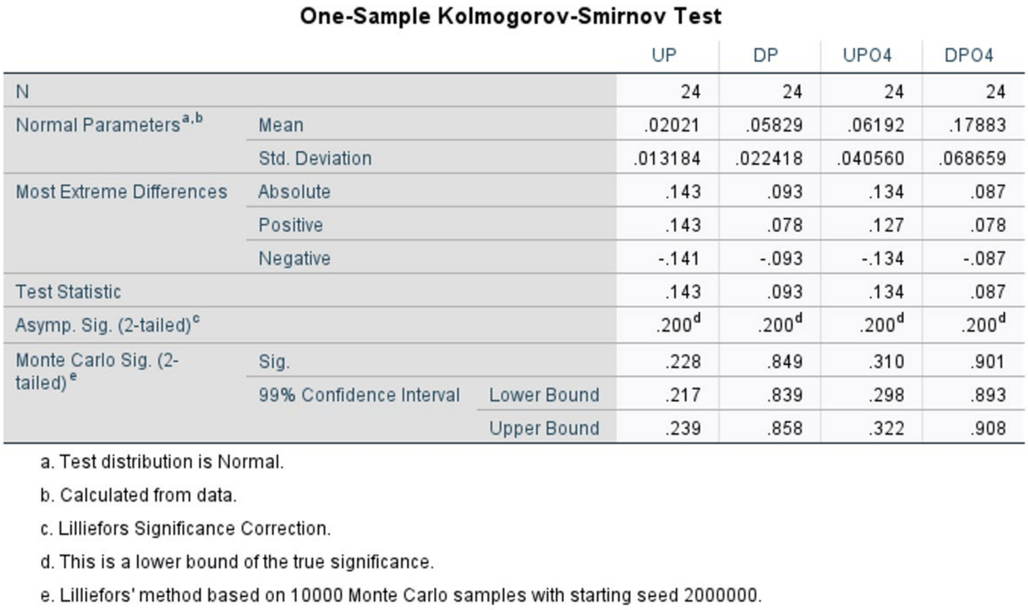
8. Appendices

Appendix A. UKTAG WFD annual mean reactive P (mgL-1) thresholds in UK rivers. Note that high altitude >80m and high alkalinity >50mgL-1 CaCO3.

River Type	Annual mean of reactive phosphorous (mg L^{-1})			
	High	**Good**	**Moderate**	**Poor**
High altitude, low alkalinity	0.013 (0.013-0.020)	0.028 (0.028-0.041)	0.087 (0.087-0.117)	0.752 (0.752-0.918)
High altitude, high alkalinity	0.024 (0.018-0.037)	0.048 (0.028-0.070)	0.132 (0.109-0.177)	0.898 (0.829-1.012)
Low altitude, low alkalinity	0.019 (0.013-0.026)	0.040 (0.028-0.052)	0.114 (0.087-0.140)	0.842 (0.752-0.918)
Low altitude, high alkalinity	0.036 (0.027-0.050)	0.069 (0.052-0.091)	0.173 (0.141-0.215)	1.003 (0.921-1.098)

Appendix B.

Table A. SPSS output table of the Kolmogorov-Smirnov Test for concentrations of reactive P and PO_4^{3-} between S1 (U) and S2 (U).



One-Sample Kolmogorov-Smirnov Test

			UP	DP	UPO4	DPO4
N			24	24	24	24
Normal Parameters[a,b]	Mean		.02021	.05829	.06192	.17883
	Std. Deviation		.013184	.022418	.040560	.068659
Most Extreme Differences	Absolute		.143	.093	.134	.087
	Positive		.143	.078	.127	.078
	Negative		-.141	-.093	-.134	-.087
Test Statistic			.143	.093	.134	.087
Asymp. Sig. (2-tailed)[c]			.200[d]	.200[d]	.200[d]	.200[d]
Monte Carlo Sig. (2-tailed)[e]	Sig.		.228	.849	.310	.901
	99% Confidence Interval	Lower Bound	.217	.839	.298	.893
		Upper Bound	.239	.858	.322	.908

a. Test distribution is Normal.

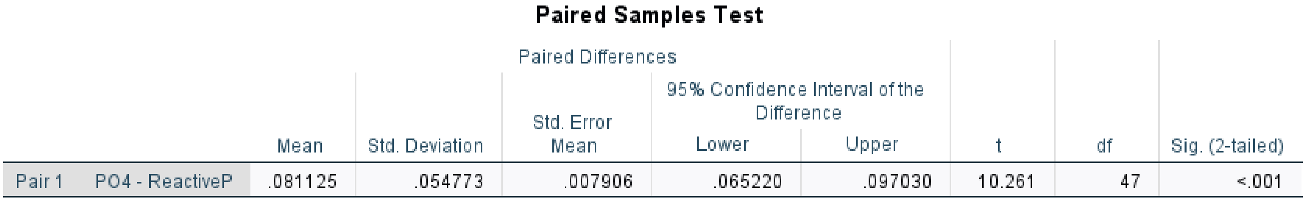
b. Calculated from data.

c. Lilliefors Significance Correction.

d. This is a lower bound of the true significance.

e. Lilliefors' method based on 10000 Monte Carlo samples with starting seed 2000000.

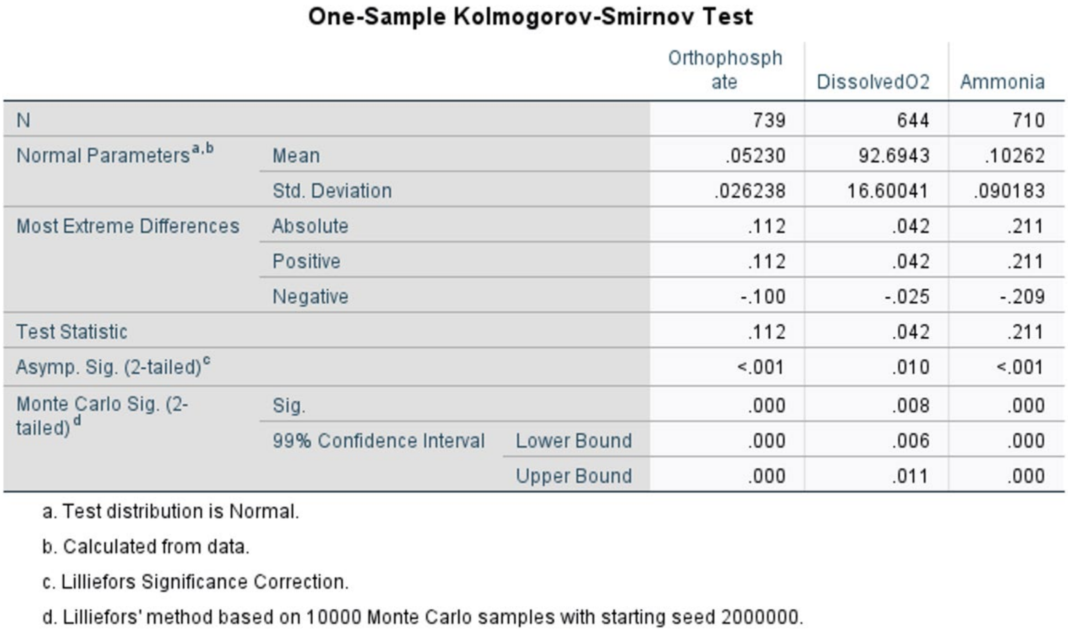
Table B. SPSS output table of the Paired-Samples-T-Test for concentrations of reactive P and PO_4^{3-} between S1 (U) and S2 (U).



Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	PO4 - ReactiveP	.081125	.054773	.007906	.065220	.097030	10.261	47	<.001

Table C. SPSS output table of the Kolmogorov-Smirnov Test for monthly samples of water quality parameters between EA1-3.



One-Sample Kolmogorov-Smirnov Test

			Orthophosphate	DissolvedO2	Ammonia
N			739	644	710
Normal Parameters[a,b]	Mean		.05230	92.6943	.10262
	Std. Deviation		.026238	16.60041	.090183
Most Extreme Differences	Absolute		.112	.042	.211
	Positive		.112	.042	.211
	Negative		-.100	-.025	-.209
Test Statistic			.112	.042	.211
Asymp. Sig. (2-tailed)[c]			<.001	.010	<.001
Monte Carlo Sig. (2-tailed)[d]	Sig.		.000	.008	.000
	99% Confidence Interval	Lower Bound	.000	.006	.000
		Upper Bound	.000	.011	.000

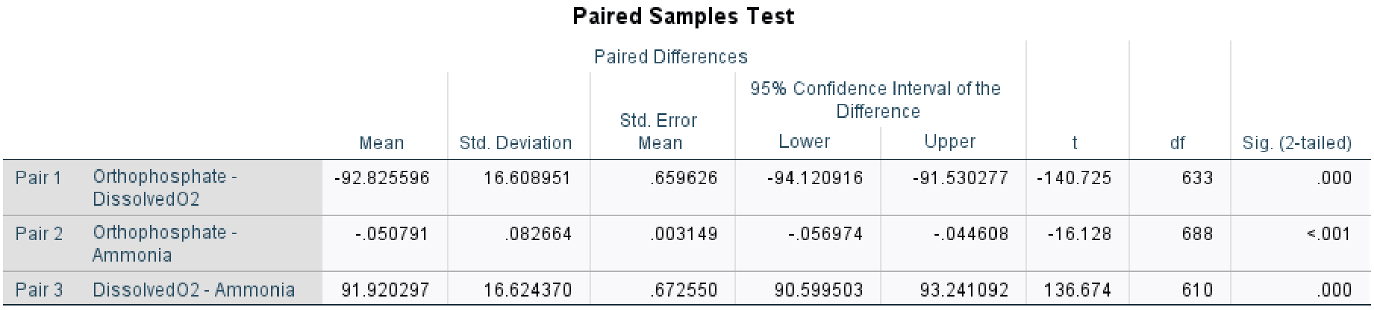
a. Test distribution is Normal.

b. Calculated from data.

c. Lilliefors Significance Correction.

d. Lilliefors' method based on 10000 Monte Carlo samples with starting seed 2000000.

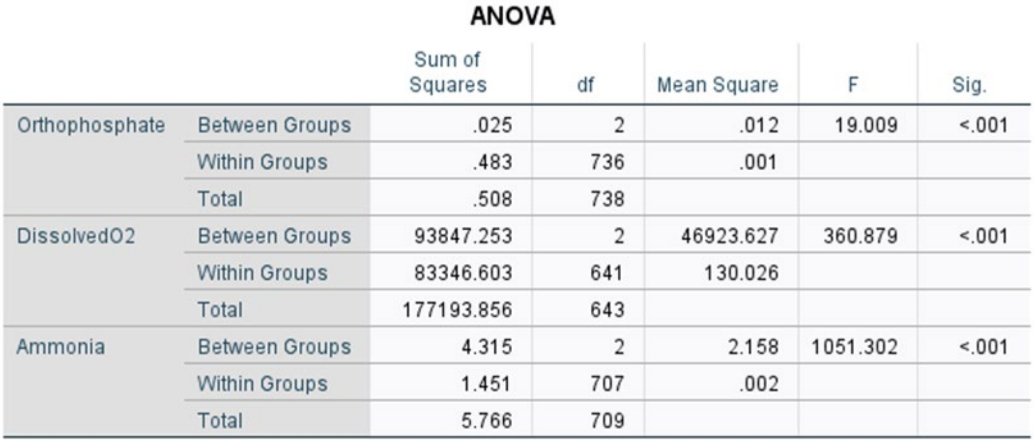
Table D. SPSS output table of the Paired-Sample-T-Test for monthly samples of water quality parameters between EA1-2.



Paired Samples Test

		Paired Differences							
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
Pair 1	Orthophosphate - DissolvedO2	-92.825596	16.608951	.659626	-94.120916	-91.530277	-140.725	633	.000
Pair 2	Orthophosphate - Ammonia	-.050791	.082664	.003149	-.056974	-.044608	-16.128	688	<.001
Pair 3	DissolvedO2 - Ammonia	91.920297	16.624370	.672550	90.599503	93.241092	136.674	610	.000

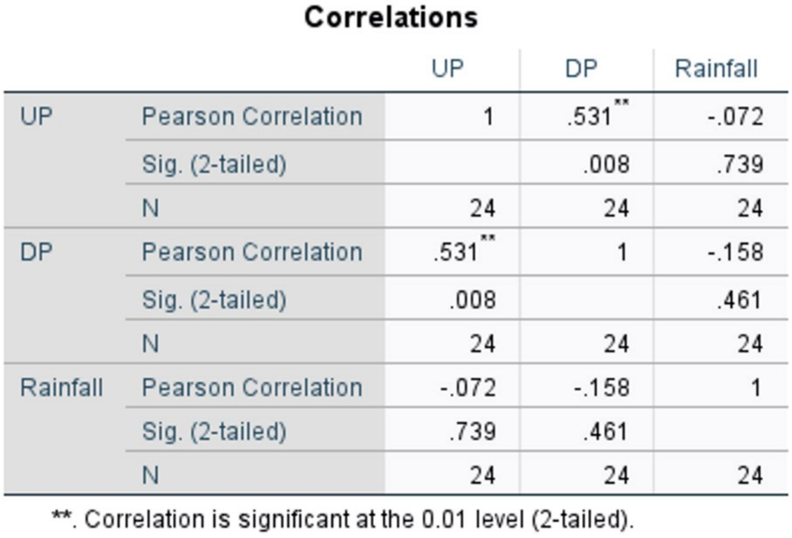
Table E. SPSS output table of the One-Way-ANOVA-Test for monthly samples of water quality parameters between EA1-3.



ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Orthophosphate	Between Groups	.025	2	.012	19.009	<.001
	Within Groups	.483	736	.001		
	Total	.508	738			
DissolvedO2	Between Groups	93847.253	2	46923.627	360.879	<.001
	Within Groups	83346.603	641	130.026		
	Total	177193.856	643			
Ammonia	Between Groups	4.315	2	2.158	1051.302	<.001
	Within Groups	1.451	707	.002		
	Total	5.766	709			

Table F. SPSS output table of the Pearson's Correlation Coefficient between diurnal concentrations reactive P at S1-2 and daily HadUKP rainfall data over the 24-day trial period.

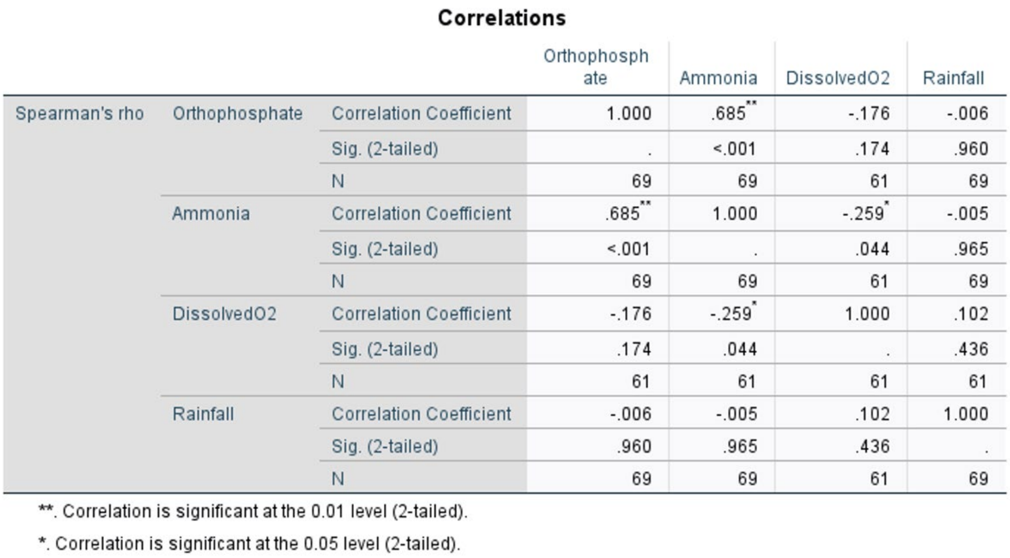


Correlations

		UP	DP	Rainfall
UP	Pearson Correlation	1	.531**	-.072
	Sig. (2-tailed)		.008	.739
	N	24	24	24
DP	Pearson Correlation	.531**	1	-.158
	Sig. (2-tailed)	.008		.461
	N	24	24	24
Rainfall	Pearson Correlation	-.072	-.158	1
	Sig. (2-tailed)	.739	.461	
	N	24	24	24

**. Correlation is significant at the 0.01 level (2-tailed).

Table G. SPSS output table of the Spearman's Rank between annual averages of water quality parameters at EA1-2 and annual average HadUKP rainfall data from 2000-2022.



Correlations

			Orthophosphate	Ammonia	DissolvedO2	Rainfall
Spearman's rho	Orthophosphate	Correlation Coefficient	1.000	.685**	-.176	-.006
		Sig. (2-tailed)	.	<.001	.174	.960
		N	69	69	61	69
	Ammonia	Correlation Coefficient	.685**	1.000	-.259*	-.005
		Sig. (2-tailed)	<.001	.	.044	.965
		N	69	69	61	69
	DissolvedO2	Correlation Coefficient	-.176	-.259*	1.000	.102
		Sig. (2-tailed)	.174	.044	.	.436
		N	61	61	61	61
	Rainfall	Correlation Coefficient	-.006	-.005	.102	1.000
		Sig. (2-tailed)	.960	.965	.436	.
		N	69	69	61	69

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).