Simple Tools for Improved Management of Small Wastewater Treatment Plants

Joshua Thomas Bunce BSc (Hons)

School of Engineering

Faculty of Science, Agriculture and Engineering

Newcastle University

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Abstract

The treatment performance of small WWTPs (< 250 PE) in England is not well understood and their ecological impact may be underestimated. However, the critical role such systems play in ensuring sustainable wastewater management, means they can no longer be neglected. The aim of this thesis, therefore, was to provide new data, understanding and analytical approaches to improve the management of existing, small WWTPs. Firstly, through an extensive sampling campaign, we found a significant difference ($p < 0.05$) between the effluent quality discharged from twelve small and three larger WWTPs across a range of abiotic parameters. Specifically, mean removal rates at the small plants were 67.3 ± 20.4%, 80 ± 33.9% and 55.5 ± 30.4% for sCOD, TSS and NH_4-N (± standard deviation), respectively, whereas equivalent rates for larger plants were 73.3 ± 17.6%, 91.7 ± 4.6% and 92.9 ± 3.7%. A Random Forest classification model accurately predicted the likelihood of a small WWTP becoming unreliable. Among the important predictors was population equivalence, suggesting the smallest WWTPs may require particularly stringent management. Quantifying, in the raw and treated wastewater samples, three genetic faecal markers targeting *Bacteroides* and two targeting *E. coli*, revealed that human-associated *Bacteroides* markers have the greatest potential as alternative performance metrics at small WWTPs, however, all markers were influenced by seasonality. Next, the problem of predicting flows at small scales was overcome using an inverse approach to solve a linear reservoir function (NSE = 0.77 – 0.93). The model was combined with the field data to generate pollutant loads and investigate the effect of influent peak loading of COD on the final effluent quality at small discharges. Simple tools developed, here, provide wastewater managers with new techniques to improve the operation and increase the understanding of small WWTPs. Growing awareness of the need for sustainable wastewater and water resources management makes the work both timely and of global relevance.

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Chapter 1. Introduction

Wastewater management infrastructure accounts for approximately 1% of global gross domestic product (Ashley & Cashman, 2007) and is central to public and environmental health. Considering also, the geopolitical complexity surrounding the growing demand for water resources, the effective treatment of wastewater has never been a greater priority. However, globally, there is an overreliance on aging wastewater infrastructure which Eggimann et al. (2018) suggest leads a conservative industry to technological dependence and is blocking the emergence of innovations in operational management and technology development. This is a particular concern in rural and remote areas in England. Regulatory conditions (EA, 2018b) mean that decentralised wastewater treatment plants (WWTP; Figure 1) have historically been neglected. The reduced regulatory control invokes limited management, monitoring and data which, in turn, results in an incomplete understanding of system performance and discharge impact (Istenic et al., 2015; Eggimann et al., 2017). Thus, there is an over dependence not only on traditional technologies, but also traditional understanding that could be derived from data collected at centralised systems that do not accurately reflect the behaviour or form of their smaller counterparts.

The transition to more sustainable wastewater management has primarily focussed on centralised assets through the application of such innovations as natural gas production from the anaerobic digestion of wastewater sludge and minimised energy consumption. However, an exclusive focus on centralised infrastructure is of limited benefit and a mix of well-managed, decentralised and centralised investment is essential for long-term sustainability (Eggimann et al., 2018). The economic benefits of this approach become apparent when considering the institutional capacity and financial commitment required to support a large and complex infrastructure (Sadoff et al., 2015). Such attributes have been a barrier to the centralised sewer connection rates in non-OECD countries in particular (Sadoff et al., 2015), which remain low. However, the poor reputation of traditional, small-scale WWTPs is perhaps also to blame for limited progress towards achieving the ambitions of Sustainable Development Goal 6 (McDonald et al., 2014; United Nations, 2018). Therefore, the importance of improved understanding and management of existing, small-scale wastewater systems is timely and of global relevance.

Figure 1 - Examples of small WWTPs in NE England. Clockwise from top-left, technologies are trickling filter, high performance aerated filter, rotating biological contactor and activated sludge.

Knowledge gained from the development of centralised wastewater management, whilst perhaps of limited bearing to small scales, should not be discounted. It has contributed to step-changes in public health improvement through the eradication of diseases such as cholera, and ecological improvements that have increased the amenity and accessibility of watercourses. However, there is a clear need to translate this understanding to smaller systems and develop tools specifically for small-scale applications. One such example is related to treatment performance metrics. In England and elsewhere, final effluent discharge regulations are predominantly driven by the potential for adverse ecological impact and do not typically apply to small-scale systems (EA, 2018b). With growing interest in decentralised water reuse (Wilcox et al., 2016; Leong et al., 2017; Jonasson & Kandasamy, 2017) and the key role small-scale systems might play in addressing sanitation problems in non-OECD countries (Graham et al., 2019), it is important to consider alternative treatment performance metrics. Recent advances in genetics has allowed the development of rapid, highly specific molecular techniques for aiding the quantification of water pollution health risks. Such techniques present a new opportunity for health-driven wastewater management that might be particularly useful for assessing and even designing, small-scale WWTPs.

This thesis describes the development and application of simple, mathematical tools and analysis approaches to improve the management of small WWTPs, with a specific focus on rural areas in North-East (NE) England. The geographical region of interest covers the Northumbrian Water Ltd. wastewater operational area, which extends from the England-Scotland border in the North to the North Yorkshire Moors in the South; from the East coast of England to Alston, Cumbria in the West. Simple tools have been chosen, specifically, to encourage adoption, by wastewater managers, of the assessment techniques demonstrated in this thesis. The word 'simple' in this context refers to the conceptual theory underpinning the analysis approach. For example, the use of a lower number of predictor variables in numerical models. Such a philosophy has been applied extensively across a broad range of fields, including economics, genetics and geophysics (Barro, 1988; Shiraishi et al., 2015; Braun et al., 2016). It is particularly appealing for use in informing the management of small WWTPs because of the often-limited data requirements of simple models.

1.1 Aims and Objectives

The aim of this study, therefore, was to provide new data, understanding and analytical approaches to improve the management of existing, small WWTPs. The aim was met by fulfilling the following objectives:

1. Improve understanding of the effect of scale and technology type on the performance and stability of small WWTPs.
2. Evaluate the potential of genetic faecal markers for assessing small WWTPs and thereby, provide insight into the potential impact of their discharges on upper catchment water quality.
3. Evaluate the influence of wastewater flow rate characteristics on the treatment performance of small WWTPs.

1.2 Thesis Structure

The thesis consists of seven chapters, including this Introduction. Chapter 2 is a literature review which provides the reader with sufficient knowledge to interpret the presented research. The review includes a summary of important and recent literature relating to, different types of small WWTP, the use of genetic faecal markers as health-risk indicators and, the prediction of wastewater flow rates. This is followed by the presentation of findings from four studies. Firstly, an extensive sampling programme provided data on the treatment performance of twelve small WWTPs and facilitated the development of a simple, reliability prediction methodology. Using DNA extracted from the same samples, genetic faecal markers were quantified and proposed as an alternative treatment performance metric for small WWTPs. Chapter 5 describes the development of a flow prediction tool to overcome the lack of flow monitoring data at most small WWTPs. The final research study draws together Chapters 3, 4 and 5, by applying the flow prediction model to assess the impact of flow characteristics on treatment performance. Finally, the thesis is concluded in Chapter 7.

Chapter 2. Literature Review

2.1 Defining Small-scale Wastewater Treatment

Small-scale, decentralised wastewater treatment involves the collection and treatment of wastewater close to the point of production (Crites & Tchobanoglous, 1998). Definitions of 'small' are inconsistent across literature and usually chosen for relevance to local context which may be driven by regulation or the availability of different technologies. For example, in 2003, the European Commission defined decentralised WWTPs as being less than 5000 population equivalent (PE; Berland et al., 2003). Whereas, the European Committee of Standardization defined 'small' as applying only to WWTPs serving less than 50 PE (CEN, 2005). Gutterer et al. (2009) and Wendland & Albold (2010) chose an upper limit defined by volume, specifically 1000 m^3 wastewater treated per day, and more recently, Roefs et al. (2017) define 'neighbourhood-scale' treatment systems as being between 600 and 1200 PE in their economic evaluation of centralised, decentralised and hybrid sanitation systems. The result of this inconsistency is that using literature to inform wastewater management strategies that consider small-scale technologies is complex and difficult. Interestingly, in England, the regulatory authorities do not draw a clear distinction between centralised and decentralised WWTPs based on size alone, but consider, also, the ecological impact of the final effluent discharge, irrespective of system size (EA, 2018b). Furthermore, van Afferden et al. (2015) suggest that the use of the term 'decentralised wastewater management' should only be in reference to the distance from the point of production, which is consistent with that of Crites & Tchobanoglous (1998). Perhaps, this draws in to question the appropriateness of defining WWTPs by scale all together, which is something that this thesis explores.

For the purposes of this thesis, 'small' is defined by regulatory guidance for England which indicates that all continuous wastewater discharges of less than 50 m^3/day are exempt from numerical regulation, including flow rate monitoring (EA, 2018b). As stated, the exception being where effluent discharges to an ecologically sensitive water course targeted for improvements or limiting deterioration under legislation such as the Water Framework Directive (WFD) (EC, 2000). Where flow data are not available, PE is often used as a proxy for treatment volumes. In the UK, 250 PE equates to a treated flow of approximately 50 m^3/day. For consistency, this approach has been adopted throughout the thesis.

2.2 The Case for Decentralisation

Small-scale WWTPs should not be viewed as an alternative to centralised infrastructure, but rather, complementary to it (Van Afferden et al., 2015). In some instances, the use of small-scale WWTPs might be out of necessity. For example, where building sewers are considered uneconomical. However, there is a case to be made for the preferential use of small-scale WWTPs, particularly in regions experiencing rapid urbanisation (Wang, 2014).

Traditionally, economies of scale have favoured centralised treatment which has been reflected in urban planning (Tihansky, 1974). However, the cost-benefit of centralised wastewater treatment has been called into question on several accounts. Firstly, because the majority of capital investment can be attributed to sewerage infrastructure, which may be up to 90% of the total capital cost (Maurer et al., 2005). The cost-benefit may, in reality, be a result of high population density, which would not necessarily be a benefit exclusive to centralised systems. In other words, if high population density minimises the length of sewerage infrastructure required, this applies regardless of whether the treatment facility is centralised or decentralised. Secondly, population growth forecasts up to thirty years in advance (as is typical) can lead to idle treatment capacity of up to 50% (Maurer, 2009). Evidently, this requires a large capital outlay to accommodate forecasting risk, which may be even greater at times of global economic uncertainty. Thus, money becomes tied up in the idle capacity of centralised assets. Conversely, when wastewater management is decentralised, the incremental development of infrastructure could negate the need for idle capacity. Wang (2014) improved the work of Maurer et al. (2009) by showing the effect of idle capacity on the net present value of a WWTP. The author demonstrated how under most circumstances, capital investment in decentralisation can be justified on the basis of cost saving by reducing idle capacity, even though the capital cost per PE might be greater than for a centralised WWTP.

A common criticism of small WWTPs is that the management of them becomes the responsibility of the local community, or asset owners (in the case of a hospital or university, for example). Given the obvious requirement for technical expertise to carry out effective maintenance and ensure regulatory compliance, centralised management of decentralised WWTPs has been proposed on a number of occasions (Massoud et al., 2009; Jorsaraei et al., 2014). Gikas & Tchobolangous (2009) highlight the benefits of such an approach and while their review specifically considers water reclamation, many of the principles apply. The authors demonstrate how a hybrid strategy could mitigate capacity limitations, account for increased population growth, and address water quality issues through centralised management; all factors of relevance to any wastewater management system. Additional benefits to a decentralised approach to wastewater management, which has been recognised in recent years, include: facilitating localised wastewater reuse (Tchobanoglous et al., 2004; Brown et al., 2010) and resource recovery (Ho, 2005; Hong et al., 2005; Ronteltap et al., 2007; Weber et al., 2007; Borsuk et al., 2008) smaller physical footprint and reduced aesthetic impacts (Brown et al., 2010).

Whilst there are evidently benefits to decentralising wastewater management, or incorporating small-scale systems into an existing set-up, the performance of such systems must be considered relative to their larger counter-parts. Making comparisons between different small-scale technologies is difficult because of inconsistencies in experimental design, analytical methods and system size and generally, the lack of performance data across a range of metrics (Bunce et al., 2018). Therefore, an aim of this thesis was to fill the data gap and present a robust comparison between different types of small WWTP. By way of background, a summary of traditional, novel and emerging technologies follows.

2.3 Small Wastewater Treatment Technologies

This section provides a brief overview of the wastewater treatment technologies typically employed in the UK, including those assessed in this study. The prevalence of particularly technologies is likely to be largely historical and varies between and within UK water company operating areas. The national asset base is dominated by trickling filter systems and this also is reflected at small scales, however other technologies also are prevalent. Recently, more sophisticated options have been chosen to comply with regulatory targets and/or achieve ecological ambitions. Such technologies, which at small scales typically are package plants, are of growing interest and so are considered, here.

The intention is to provide sufficient information to effectively interpret the study; the intention is not, therefore, to provide an exhaustive review of all available literature relating to the technologies. However, key manuscripts have been considered and are used to summarise the history, recent development, and treatment performance trends of different types of small WWTP.

2.3.1 Septic tanks

Septic tanks are generally regarded as the most rudimentary treatment system and yet widely considered the ‘standard’ for single-household wastewater treatment. Despite being over 120 years old, the technology remains generally unchanged and systems can range in size from 1 PE to 200 PE or larger (Siegrist, 2017). Briefly, wastewater flows into a concrete tank and is ‘treated’ by settling of particulate matter, which is then degrades anaerobically. A two-stage version of the septic tank, known as the Imhoff Tank, separates the degradation step (i.e., digestion) from solids settling. Although common in emerging and developing countries, new installations of the Imhoff Tank have largely been phased out in the UK since the 1950s.

Several recent studies have attempted to quantify the potential impact of septic tank effluent discharges on surface water quality in the UK. their study of 32 septic tanks in Scotland. Final effluent concentrations of chemical oxygen demand (COD) ranged from 48 - 5514mg/L, ammonium (NH_4-N) ranged from 0.03 – 144 mg/L and total phosphorus (TP) ranged from 0.2 – 32.5 mg/L (Withers et al., 2011, 2012; Richards et al., 2016). These studies highlight the common problem of highly variable treatment performance which may be linked to operational maintenance (e.g., sludge removal) or incorrect sizing. Whilst the septic tanks assessed were generally for single occupancy properties, the performance ranges also apply for larger systems (Siegrist, 2017).

Various attempts have been made to enhance or modernise the septic tank. Features including the simple addition of baffle systems (Nasr & Mikhaeil, 2015) or complex adaptation to enhance the functionality of the system beyond simply treating wastewater (e.g., methane for a combine heat and power plant; (Park, 2015). In a recent example, baffling was used to create a multi-chamber system and intermittent aeration was provided to create an aerobic zone (Abbassi et al., 2018). The authors report mean COD effluent concentrations of 88 (± 35) mg/L and mean effluent NH_4-N of 39.8 (± 22) mg/L from pilot-scale systems operating under a hydraulic loading rate of 2 m^3/day. Whilst the average treatment performance is encouraging, operational variability is wide and the relative economic or environmental value of modifying an aging septic tank versus replacing the system with a similarly configured package plant, must be called into question.

In the UK, the General Binding Rules: Small Sewage Discharge to a Surface Water, state that, "discharges from [privately owned] septic tanks directly to a surface water are not allowed" (EA, 2018a). Instead, septic tanks must be replaced by package treatment plants. Water and wastewater companies in the UK are not required to fulfil the same obligations. In contrast, in the USA, no septic tank effluent can be discharged into a ditch, stream, lake or ocean without additional treatment, regardless of ownership (Siegrist, 2017).

2.3.2 Constructed wetlands

Constructed wetlands rely on the replication of processes that occur in natural wetlands and can result in the degradation of pollutants by chemical, biological and physical means (Castellar et al., 2018). They generally provide better treatment performance than a natural wetland because the hydraulic regime can be engineered and is more easily controlled (Polprasert, 2004). Their low aesthetic impact, relatively low cost and simple operation are reasons for their growing appeal as sustainable solutions for rural wastewater treatment (Nivala et al., 2013, 2019). Variations in system design range from simple, passive horizontal flow to highly engineered and complex systems that involve pumping and mechanical aeration (Fonder & Headley, 2016). The development of different systems has been inter-disciplinary and international, which has led to a plethora of design specifications, driven by local regulatory requirements for planning and ecological protection (Nivala et al., 2013). Therefore, there are no internationally adopted design standards which makes comparison between systems difficult. Wetlands are particularly common for individual houses or clusters of properties. For example, in Austria, 40% of WWTPs serving communities of less than 50 PE installed since 2000 are constructed wetlands (Langergraber & Weissenbacher, 2017). Interestingly, installation numbers peaked in 2011, which may have been a result of achieving regulatory compliance; the reason is unclear.

Traditional constructed wetlands consist of water-tolerant vegetation grown in gravel and/or sand through which wastewater flows horizontally on or below the surface (i.e., free-flowing or horizontal sub-surface flowing) (Wu et al., 2014). In contrast, more recently developed vertical sub-surface flow wetlands operate by the wastewater being ‘evenly’ distributed across or below the surface of the wetland and subsequently treated by passing vertically through the vegetation and filter bed structure. Particulate matter in the wastewater is removed by deposition and filtration, which over time, will result in a layer of sludge forming on the wetland surface (Polprasert, 2004). Soluble organic matter is removed by microbial activity, and likewise for the majority of nutrient removal.

Recent developments have improved or tailored treatment performance by the addition of: artificial aeration to increase oxygen concentrations in the wastewater (Butterworth et al., 2013; Zhang et al., 2010; Ilyas & Masih, 2017); baffling to allow better control of hydraulics (Tee et al., 2012); earth worms to increase nutrient and carbon (Xu et al., 2013); and stacking wetlands vertically as a space-saving solution (Ye & Li, 2009). The use of artificial aeration is the most widely studied adaptation and has resulted in increased removal of faecal indicator organisms as well as abiotic parameters (Uggetti et al., 2016). Specifically, in their UK-based study, the authors found that artificial aeration reduced NH_4-N effluent concentrations from 6.75 mg/L to 0.15 mg/L, although it had very little effect on COD concentrations. The associated energy and maintenance costs of artificially aerating a constructed wetland must be considered because the technology is specifically designed to offer an environmentally sustainable and aesthetically pleasing alternative to traditional wastewater treatment. In all cases, treatment by constructed wetlands require prior separation of solids which may make them suitable for treating septic tank effluent but means that they do not generally provide a ‘stand-alone’ treatment solution. In NE England, wetlands are used most commonly as a tertiary treatment step to remove specific pollutants (e.g., nutrients) prior to discharge into an ecologically sensitive watercourse.

2.3.3 Rotating Biological Contactors

The modular structure of package treatment plants makes them an ideal option for decentralised wastewater management. Rotating Biological Contactors (RBCs) evolved from the development of a paddle-wheel style device, first reported in the early 20th Century (Allen, 1929); however, it was not until the 1960s that RBCs began to be installed in Europe. In the 1970s, the modern iteration incorporating corrugated media became commercially available. The majority of installations are for communities of less than 1000 PE, which highlights its appeal for small-scale applications (Antonie, 2017). In the early 1990s, RBCs were recommended as the WWTP of choice for small communities by (Greaves et al., 1990), who noted the low operational maintenance requirements and reliable performance relative to extended aeration package plants and more efficient contact times compared to trickling filters. However, the conclusion was drawn by comparing just the two technologies, and ‘low maintenance’ was defined as at least three site visits per week, which is not objectively low. The questionable reliability of such findings highlights the need for further studies on the localised performance of RBCs compared to different technologies.

RBC reactors consist of a continually rotating disc that is approximately 40% submerged in wastewater and acts as a support matrix for biofilm growth (Figure 2). The system is particularly effective because it employs the benefits of passive aeration utilised by a conventional trickling filter and also aeration provided by the rotating wheel, oxygenating the wastewater when the wheel is partially submerged. As the discs rotate, they lift a thin film of wastewater that falls under gravity across the biofilm, permitting biodegradation and removing organic matter (Antonie, 2017). Further removal occurs via the suspended biomass, which is aerated by the rotating discs.

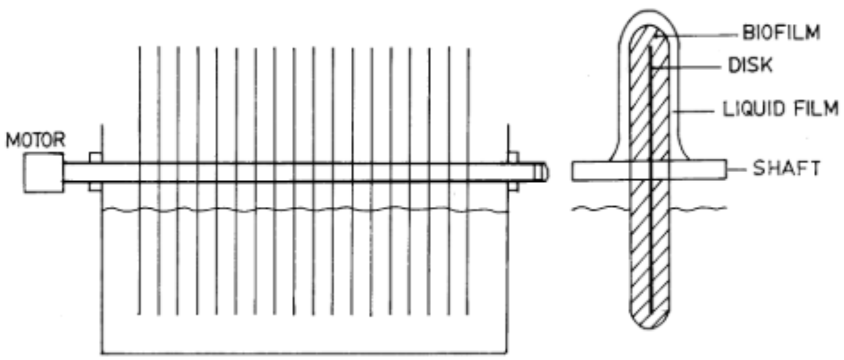


Figure 2 - Schematic of a single rotating biological contactor unit (Patwardhan, 2003).

Although effective at many levels, RBCs are rather sensitive to the organic loading rate (OLR). Hiras et al. (2004) reported a decrease in COD removal from 50 to 35%when the OLR was increased from 90 to 360 g m^{-2} d^{-1}. This may be explained, in part, by oxygen transfer rate limiting substrate uptake efficiency by the biofilm (Di Palma & Verdone, 2009), which is important because the effectiveness of an RBC is usually more dependent on the physical mass transfer capacity of the biofilm rather than biological kinetics limitations (Hassard et al., 2015). In their review, the authors also note that under constant loading, the biofilm and suspended microbial community readily attain steady state based on the consistent substrate availability. In contrast, under variable hydraulic and loading conditions, the microbial community can become unstable due to starvation, excess sloughing, or washout.

RBCs also have the potential to discharge effluent with a particularly high concentration of suspended solids, especially in UK summer. Higher atmospheric temperatures accelerate biofilm growth (Madigan et al., 2012), which may be more easily stripped from the support media by shear stresses caused by the passing wastewater, which is particularly common due to the vertical orientation of the discs. It has been suggested this stripping phenomena prevents clogging of the media and the agitation of the bulk liquid caused by the continually rotating discs means that the stripped biomass stays in suspension (Antonie, 2017). There are two circumstances under which this benefit might not be realised: 1) at very small-scales (less than 250 PE, for example), which is when WWTPs are subject to a particularly high peaking factors, and 2) under storm conditions that cause sudden peaks in wastewater flux at the WWTP. In both scenarios, a sudden increase in shear stress would likely cause greater stripping of biomass. Furthermore, the capacity of the bulk liquid to maintain solids in suspension is finite and, especially at higher temperatures, which is when excessive microbial growth also would occur in the liquid.

Improvements to the performance of RBCs, in terms of pollutant removal, have long been understood and can be made by increasing the speed of rotation of the disks (Friedman et al., 1979), varying support media material (Hassard et al., 2014), or by adjusting the number of treatment stages which can lead to a step-wise reduction in substrate (Antonie, 2017). Furthermore, staging can act to buffer some of the negative effects of shock-loading (Hassard et al., 2015). However, this has not been investigated on a diurnal basis in full-scale systems treating real wastewater. Interestingly, recirculation of effluent or solids does not appear to dramatically improve reactor performance (Wilson & Lee, 1997; Klees & Silverstein, 1992). Whilst recirculating effluent might result in a slight increase in nitrification due to a lower overall carbon concentration, this often is at the expense of COD removal, which is undesirable. Few studies have tested such performance improvements beyond laboratory or pilot-scale and/or using variable strength domestic wastewater; therefore, drawing meaningful comparisons is difficult. A single stage RBC might expect to achieve final effluent COD concentrations of 75 mg/L and NH_4-N concentrations as low as 2 mg/L (Tchobanoglous et al., 2003).

2.3.4 Biological Aerated Filters

Biological Aerated Filters (BAFs) are fixed-film reactors typically used for secondary or tertiary wastewater treatment (Stensel et al., 1988). The media may be suspended or fixed in an aerated unit, but also can be applied in anaerobic systems. The high specific surface area of the media provides a greater biomass concentration and also has the effect of separating the solids retention from the hydraulic retention (Mann et al., 1999), which allows greater operational control (Tchobanoglous et al., 2003). There are numerous commercially available BAFs, which employ different media types and vary in configuration. A recent review (Heinonen-tanski & Matikka, 2017) analysed 717 effluent samples collected from small WWTPs in Finland, including many commercially available technologies. The authors drew an important conclusion: that simple, locally constructed sand filters can produce effluents that are at least as good quality as commercially available package plants. Part of their reasoning is that due to their complexity most modern package plants require frequent attention and are often treated as ‘black-boxes’ by users who may not fully understand the technology. This is undoubtedly a useful observation, but it is specific to the Finnish context and their sand filter systems. Also, the samples numbers upon which the data were based ranged between four and ninety-seven per treatment technology, calling into question the statistical validity of the analysis. Furthermore, no details are reported on configurations, asset ages or unit operations of individual WWTPs. This lack of experimental design means the study is more useful for assessing general trends in small WWTP performance in Finland than for drawing general conclusions and making recommendations regarding specific technologies. None-the-less, the dataset is almost unique amongst publicly available literature in comparing traditional and package technologies in a European context and so provides, at the very least, a summary of different types of WWTP. This thesis will seek to fill the data gap for traditional and modern technologies in the UK and, specifically, NE England.

Examples of commercially available BAF systems exist. The DELPHIN Cube incorporates a fixed-bed biofilm support matrix and membrane diffuser to aerate the bulk solution (DELPHIN, 2019). In contrast, the Biokube® utilises a series of fixed filter modules, reported to achieve up to 82% COD removal and 84% NH_4-N removal when operated at a hydraulic retention time of 22 hours using four modules (Choi et al., 2015). Finally, the BioWater Technology CFIC® is a second generation moving bed bioreactor that includes the tight packing of biofilm carriers to minimise movement, which is suggested by the manufacturer to negate the need for final clarification, thus improving effluent quality (BLUEWATER BIO, 2019). With the exception of BioKube, which has been adopted extensively across the world (7000 installations at the time of writing; BIOKUBE, 2019), most commercially available technologies published reports of extensive, rigorous analysis of treatment performance are not available, especially compared to alternative technologies. Consequently, there are also minimal, independently tested treatment performance data on such technologies. This poses a major problem for the UK water industry (and elsewhere) where comparative data is lacking for identifying “most suitable” technologies for rural and decentralised applications.

For the purposes of this thesis, a commercially available BAF of particular interest is the High-Performance Aerated Filter (HiPAF) developed by WPL International (WPL, 2019). HiPAFs are commonly used in NE England, especially where there is a regulatory requirement to discharge high-quality effluent to surface waters. The technology incorporates a primary settlement, a combined fixed film and dispersed floc active unit and a secondary clarifier, within a modular system (Figure 3). Unlike several other BAFs (Tchobanoglous et al., 2003), the HiPAF includes a sludge return system to ensure longer solids retention.

The HiPAF system is designed to minimise aesthetic impact, which may be important in rural areas of the UK and is reported by the manufacturer to produce high-quality effluents (WPL, 2019). The use of efficient air diffusers simultaneously maintains a sufficient dissolve oxygen concentration in the bulk solution and scour the filter media. The latter is particularly important in fixed film systems in UK summer when higher temperatures increase biofilm growth (Madigan et al., 2012). The modular system is typically buried in the ground which, as well as reducing aesthetic impact, acts to insulate the process from external temperature changes that might influence treatment performance. To this author’s knowledge, there are no externally verified, comparative performance data on the HiPAF system published in scientific journals.

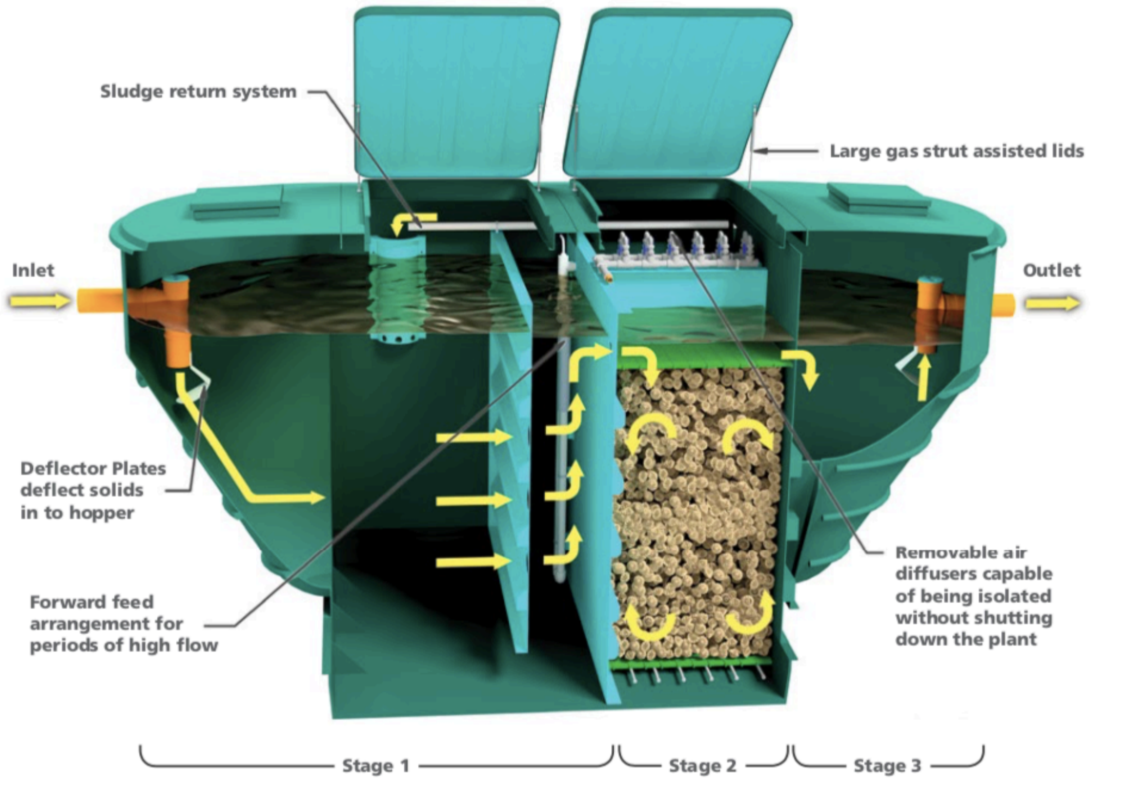


Figure 3 - Schematic of the WPL High Performance Aerated Filter (HiPAF) system WPL, (2019).

2.3.5 Other established technologies

Other traditional WWTP technologies commonly applied at small scales are derived from designs of larger counterparts. These include, conventional activated sludge, oxidation ditches, and trickling filters. The treatment configuration typically consists of a primary settlement tank, biological treatment unit and a rudimentary secondary clarifier. There have been no recent studies that consider the performance and/or potential ecological impact of traditional activated sludge or trickling filter systems serving small communities in the NE of England. This is likely because the assumption that small WWTPs possess similar characteristics (and therefore treatment performance) as their larger counterparts, or they are neglected because of their low regulatory importance. There are several other well-established technologies, some which are aimed at small-scale applications, which have not been summarised in detail because they are not prevalent at small scales in the UK, especially in NE England. However, for completeness, a brief summary of the technologies is provided, as follows.

Membrane bioreactors (MBR) have been suggested as a suitable solution for small-scale wastewater treatment (Capodaglio, 2017; Abegglen et al., 2008). The ability of an MBR to produce high-quality effluent has been shown on a number of occasions and at various scales (Capodaglio et al., 2017). Briefly, the technology utilises a suspended growth bioreactor and microfiltration technology for solids separation (Tchobanoglous et al., 2003). The resulting retention of solids eliminates the need for secondary clarification and allows operation at a higher mixed liquor suspended solids concentration, leading to less sludge production, a smaller spatial footprint and higher quality effluent. The membrane can be either immersed within the bioreactor or it can be positioned externally, and the mixed liquor is recirculated through the membrane (Judd, 2008). Some common disadvantages of MBR systems are particularly pertinent to small-scale applications.

Firstly, the issue of membrane fouling, which occurs when fine particles enter the inner pores of the membrane, resulting in a pressure loss (Tchobanoglous et al., 2003). Solutions typically involve periodic backwashing (often using chlorine), air scouring and flushing with sodium hypochlorite, or more recently, quorum-quenching bacteria (Deng et al., 2016). Such solutions add complexity, energy, and operational costs to the system; all of which should be minimised for sustainable, decentralised wastewater treatment. Finally, the issue of capital cost associated with MBRs is often prohibitive to their wide-spread adoption. Whilst, this perception might still be limiting, it is a simple matter of economics and, in due course, the cost of the technology will decrease; it is not a fundamental reason to dismiss the potential of the system.

Another membrane-based treatment technology with growing commercial appeal is the membrane aerated biofilm reactor (MABR). The system relies on the immobilisation of biofilm on a membrane surface through which air is passively or actively diffused to oxygenate the wastewater (Casey et al., 1999). The commercial potential of the technology has attracted the interest of start-ups and multi-national corporations, including OXYMEM (a spin-off from University College Dublin), Fluence Corp Ltd., and General Electric (GE). Fluence specifically target their MABR technology at decentralised applications (Fluence, 2019), whereas GE and OXYMEM primarily target centralised WWTPs. The Fluence product is containerised making it ideal for decentralised wastewater treatment and is already the solution of choice for rural locations, such as Guizhou province, China (Atkinson, 2018). OXYMEM has recently developed a retrofit device – the OxyTUBE – which shows great potential for small-scale WWTPs. The device is a small, tube containing a 'mini MABR', which can be positioned into any biological tank to provide aeration. This could be particularly suitable as a retrofit option for small conventional activated sludge units in rural locations, or where there is a desire to reduce energy costs. Whilst commercially available and tested by one UK water company (OXYMEM, 2018), no data on treatment performance have been reported in scientific journals, including comparisons with different WWTPs.

A brief comment on waste stabilisation and algal ponds is warranted because of their growing interest in the UK. Waste stabilisation ponds are a rudimentary technology typically employed in regions of low latitude, where high levels of sunlight are common and also where space is not at a premium. However, recent innovations from companies like Gurney Environmental (Gurney, 2019) means they are gaining increased attention in the UK, with the first Aero-Fac® system in NE England being installed at the time of writing. Aero-Fac® incorporates wind-powered aeration unit that 'converts' a typical pond treatment into a modular fully aerated facultative process with automated adjustments for changes in flow regime (Horan et al., 2006) (Horan et al., 2006). In the only UK-based study published in a scientific journal, reported a mean final effluent COD concentration of 61 mg/L and NH_4-N of 7.6 mg/L when using Aero-Fac® on a two-stage lagoon system in Scotland. Whilst the system achieved close to 99% ammonia removal in summer, springtime removal dropped to 20%, highlighting the seasonal dependence of such WWTPs.

In stabilisation pond systems, wastewater typically flows through a series of large, man-made ponds such that hydraulic retention times of up to 15 days are common (US EPA, 2011). Configurations typically include anaerobic and facultative ponds, although in the UK, anaerobic ponds are uncommon (Mara, 2006). Under aerobic conditions, aeration is provided by mechanical means (e.g., Aero-Fac®) and such systems share many characteristics with conventional activated sludge systems. Under facultative conditions, oxygen is generated primarily by the photosynthetic activity of algae (von Spelburg & Chernicharo, 2006).

Innovations in pond development, additional to the incorporation of novel aeration devices, include the engineering the proliferation of specific macro-fauna such as Lemna duckweed (Alvarado et al., 2008). COD and NH_4-N removals of up to 93% and 98%, respectively, have been reported when using three duckweed ponds as a post-secondary treatment (El-shafai et al., 2007). The same study found the treatment was deficient in removal of nutrients and faecal indicators (coliforms) in Egyptian winter conditions, when daily mean atmospheric temperatures do not typically drop below 14 $^{\circ}$C (WMO, 2019). For comparison, in NE England, the mean daily temperature during meteorological winter (December, January and February) between 1981 and 2010 was 6 $^{\circ}$C (Met Office, 2010).

Algal ponds and raceways also are technologies that have shown promise for use at small scales. Bunce et al. (2018) highlighted several reasons why such systems might not be suitable for UK applications. Example include, levels of sunlight are too variable to achieve consistent treatment performance, the separation of algal biomass from the final effluent is either energy intensive (requiring a large space), or solutions are still in their infancy. However, their potential elsewhere means that a plethora of algal biofilm technologies are developed and promoted, ranging from simple, single-organism biofilm sheets (Boelee et al., 2011) and to the use of algae within an osmotic membrane photobioreactor (Praveen & Loh, 2016; Achilli et al., 2009). Many such options aim to remove specific pollutants, such as phosphorus, and, therefore, may only be suitable for tertiary treatment, which is rarely required under UK regulation (EA, 2018b). There are a few commercially available algal-based wastewater treatment technologies (e.g., Clearas Advanced Biological Nutrient Recovery (ABNR®)) and some are marketed for small-scale applications. However, issues regarding the stability of the algal community and reliable treatment performance remain (Kesaano & Sims, 2014). Furthermore, the energy and maintenance costs are likely prohibitive for UK applications (Bunce et al., 2018). Algal technologies have not been extensively applied at small scales in the UK, especially in NE England.

2.3.6 Emerging technologies

Emerging technologies mainly can be categorised as either adaptations of existing technologies (e.g., septic tanks or BAFs) or nature-based systems. For example, the addition of the CFIC® technology to conventional activated sludge plants. Novel package plants will not be covered in detail here as they are typically variations on the BAF systems described in Section 2.3.4 or innovations relating to septic tanks, as described in Section 2.3.1.

Nature-based systems are growing in popularity, driven at least in part by a global awareness of environmental sustainability and the demand for aesthetic and biodiversity-promoting infrastructure (Capodaglio et al., 2017). Constructed wetlands and recent iterations thereof have been discussed in Section 2.3.2, which includes the use of particular configurations or materials to target the removal of specific pollutants.

The idea of replicating natural processes for the treatment of wastewater has been applied to develop some interesting and novel concepts. For example, the 'WETWALL' concept summarised by Castellar et al. (2018) (Figure 4). Whilst the technology is proposed primarily for the secondary treatment of grey water and hydroponic wastewater, the theory could be applied to tertiary treatment of effluents from small communities (e.g., flats or Universities), prior to discharge. The concept involves the irrigating the wastewater through a 'living wall' consisting of modular cylinders which are passively aerated and support plants chosen for their high levels of nutrient uptake. Research to date has been focussed almost exclusively on greywater and development of the technology is at proof of concept or pilot stage (Fowdar et al., 2017; Masi et al., 2016); however, it shows promise as a novel solution where discharge to sensitive watercourses might require additional treatment steps at small scales.

Figure 4 - Artist interpretation of an example vertical flow WETWALL (Castellar et al., 2018)

Another emerging technology, which may be relevant for small-scale applications, are denitrifying downflow hanging sponge (DDHS) reactors (Bundy et al., 2017; Jong et al., 2018). The system is an evolution of downflow hanging sponge (DHS) reactors. These rely on the passive aeration of wastewater as it passes through a column, within which reticulated foam acts as a support matrix for a microbial community. The DDHS reactors incorporate a section within which foam is submerged to create an anoxic environment. Bypassing a small fraction of influent wastewater to the anoxic section provides sufficient carbon to support a denitrifying community which allows total nitrogen removal rates of over 70% and COD removal rates of ~80% (Jong et al., 2018). The technology has been proven at laboratory scale and shows promise as a low-cost, low-maintenance solution, which may be applicable for decentralised locations in the UK and further afield.

Finally, whilst not strictly a novel technology, there is growing interest in the use of anaerobic bioreactors at smaller-scales (Capodaglio et al., 2017). They typically incorporate similar features as those used in BAF or moving bed biofilm reactor systems to enhance the performance of anaerobic degradation of organic compounds and nutrients (Singh et al., 2015), and so are not discussed in specific detail here.

2.3.7 Summarising small WWTP performance

When choosing a treatment technology for small-scale application, consideration must be made regarding affordability–what the purchaser is willing or able to pay – and appropriateness – the social and environmental factors associated with the installation and operation of the system (Grau, 1996). In light of ever tightening discharge standards, regulatory pressure is also becoming an important factor when comparing and contrasting different WWTPs.

A large proportion of technology development is carried out by individuals working in the private sector who may not publish the work in scientific journals, or publicly, at all (Nivala et al., 2013). The result is that the full extent of technological advancements cannot be known but, perhaps more importantly, any reported performance of commercially available products may not have been subject to the scientific rigour required to with-stand peer-reviewed publication. Therefore, the literature review here has considered only treatment performance of technologies reported in scientific journals. The one exception is for HiPAF systems, which are particularly prevalent in the NE of England, where this study was based.

Difficulties in comparing the treatment performance of different technologies arise from either: 1) inconsistencies in experimental design and technology scales or 2) inherent limitations associated with the technology. The first point is important because it is not appropriate to compare the performance, for example, of a laboratory-scale reactor treating synthetic wastewater with the performance of a full-scale WWTP treating real wastewater. Nor, would be it be appropriate to compare the treatment performance of similar WWTPs in different countries, which may be operating under different climatic conditions and with different wastewaters, and use that data to draw general conclusions about the technology. The second reason can be easily understood by considering a specific technology. For example, due to such long hydraulic retention times and the complex flow dynamics associated with large bodies of water, it can be difficult to obtain accurate removal rate data for pond-based treatment systems.

Therefore, comparing different WWTPs only using literature can be subject to inaccuracies. There is a clear need for a robust and more extensive study assessing a range of small-scale treatment systems commonly found in the same geographically area. At the time of writing, there have been no scientifically published, extensive studies comparing the treatment performance of different full-size, small-scale WWTPs in NE England. This thesis seeks to fill this data gap.

2.4 Redefining Wastewater Treatment Plant Performance

2.4.1 Genetic faecal markers as health risk indicators

The drivers of wastewater management in the UK are almost exclusively based on ecological benefit. With the exception of wastewater discharges to recognised bathing waters, effluent quality targets are determined by the potential for adverse ecological impact (EC, 1991). This is despite the protection of public health being the underpinning motivation for ‘modern’ wastewater treatment (Sedlak, 2015). Put simply, the drive to improve treatment performance has been dictated, at least in part, by a secondary incentive for its existence. This is likely because of advances in producing potable water and because of the lack of direct access to most surface waters in the UK. The latter is important because it means the route to human exposure is difficult is identify and, therefore, the relative risk impossible to accurately quantify (Bichai & Smeets, 2013). However, in the current era of sustainable and circular economies, such aspirations now should be applied to water. With this in mind, and also the growing reality of water scarcity (Gosling & Arnell, 2016), wastewater reuse becomes a necessary consideration, even in the UK. Rural locations are ideally placed to implement this concept. In areas with a disproportionately large demand for water (e.g., for agricultural purposes), the realisation of local water reuse would save costs for pumping, treatment, and storage.

An important step to determining the feasibility of such a notion is to understand the potential risks to human health associated with wastewater that is discharged from rural wastewater sources (i.e., small WWTPs). Traditional methods for identifying faecal indicator organisms, such as *E. coli*, rely on the ability to culture organisms using growth media. These methods are well-established and remain the recommended standard for assessing health risks associated with drinking, bathing and irrigation water (World Health Organisation, 2017). However, the method provides only a limited picture of human health risks because the approach is non-targeted and considers only culturable organisms. In other words, the use of culture-based methods to quantify faecal indicator organisms for human-health risk assessments is inexact because their presence does not indicate the source of the pollution; common FIOs are present in most warm-blooded mammals (Tenaillon et al., 2010). Furthermore, organisms like *E. coli* are ubiquitous in environments with no faecal pollution sources (Yamahara et al., 2007) and the abundance such organisms can be poorly correlated with pathogens, especially viruses (Harwood et al., 2005; Hörman et al., 2004). Therefore, the level of risk to human health cannot be accurately represented by only monitoring FIOs (Ahmed et al., 2016). Similar problems of a lack of source-specificity have been associated with chemical-based pollution tracking techniques (Seurinck, Verstraete, et al., 2005).

Microbial source tracking (MST) originated towards the end of the 20th Century with attempts to link isolates of *E. coli* and Streptococci with different faecal sources by ribotype or antibiotic resistance (Wiggins, 1996; Parveen et al., 1999). Reports of potential human versus animal "target" accuracy of up to 95% made way for numerous attempts to identify the dominant sources of faecal pollution in surface waters (Harwood et al., 2014). The basic premise for MST is that certain microorganisms are specific to particular hosts and that by identifying common, genetic attributes, such microorganisms can be used to detect specific pollution sources (Shanks et al., 2010). MST methods are typically used where there is a risk of human exposure to multiple sources of faecal pollution and/or where the source of faecal contamination is unknown. They are particularly useful for providing input data to quantitative risk assessments of bathing waters (Harwood et al., 2014). However, they have not been used extensively, to assess the relative contributions of pollution sources in upper, surface water catchments, which is an emerging opportunity.

MST methods can be either library-dependant or library-independent. Library-dependent methods rely on the isolation and typing for a common attribute (e.g., antibiotic resistance) of microorganisms from a range of faecal sources (i.e., different animals or humans). Library-independent methods rely on targeting a specific genetic feature, for example, a variable region the 16S rRNA gene (Bernhard & Field, 2000). Thus, a library-independent approach relies on a different target for each faecal source. In terms of the practical application, a quantitative polymerase chain reaction (qPCR) method is most common. Harwood et al. (2014) point out that the majority of recent developments have been on qPCR methods. They were, therefore, chosen for this study.

In general, MST is favoured over direct pathogen monitoring as pathogen distribution can be varied and inconsistent, making it impractical and uneconomical for the majority of circumstances. It should be noted, however, that direct pathogen monitoring still provides an optimal view of risk to human health. So much so, that in their recent study, Hughes et al. (2017) concluded that host-specific bacterial markers alone are not sufficient for determining health risk. Rather, such markers should be used in conjunction with enteric virus quantification due to their tendency to over-estimate the likelihood of gastrointestinal illness (Hughes et al., 2017). This presents a ‘catch-22’ scenario and so it has been suggested that bacterial faecal markers are used for pollution source identification and as a screening tool prior to risk assessment.

2.4.2 Markers of human faecal pollution

The majority of genetic faecal assays are bacteria-based and a large number of these target known or putative clades of *Bacteroides sp*. (Harwood et al., 2014), which was one of the first library-independent methods developed for detected human faecal contamination (Bernhard & Field, 2000). *Bacteroides* was originally targeted because it is highly abundant in the human gut and, therefore, is likely a reliable and easily detected indicator of human faecal pollution (Seurinck, Defoirdt, et al., 2005). Crucially, certain Bacteroides sp. co-evolve with the host, meaning they can be a useful tracer over time (Bernhard & Field, 2000). The human-specific marker, HF183, targets a region of the 16S rRNA gene found within *Bacteroides dorei* and has been widely used in catchment and bathing-water studies, including attempts to standardise MST procedures (Gawler et al., 2007; Cao et al., 2018; Kabiri et al., 2016; McQuaig et al., 2012; Chase et al., 2012; Wanjugi et al., 2016; Kirs et al., 2016). HF183 was originally developed by Suerinck et al. (2005) (Seurinck, Defoirdt, et al., 2005) and since, there have been several developments using different chemistries or complementary reverse sequences, each with the aim of increasing the sensitivity and specificity of the marker. Most studies report a sensitivity of HF183 to sewage of 80-100% with no clear differences reported when using SYBR and Taqman chemistry – two of the most common qPCR methodologies (Harwood et al., 2014). The original HF183 assay reported 86% sensitivity to human faeces (Seurinck et al., 2005). Several studies have recommended HF183/ BacR287 following comparisons with other human-specific bacterial markers. More specifically and relevant to this study, Mayer et al. (2016) found this assay performed most consistently of five *Bacteroides* primer sets that were tested using municipal wastewater across a range of geographical regions.

Other common human-specific *Bacteroides* markers include HumM2 and HumM3 (Shanks et al., 2009), HuBac (Layton et al., 2006), BacHum (Kildare et al., 2007), BacH (Reischer et al., 2007) and BacHuman (Lee et al., 2010)l. Amongst these, the US Environment Protection Agency developed HumM2 markers, which target a hypothetical protein, show promise, reporting 100% sensitivity to sewage and faeces, and 99.2% specificity, including in field tests (Ahmed et al., 2016). In a study testing several human-specific *Bacteroides* markers, Ahmed et al. (2009) concluded that HF183 was the most sensitive and specific to human pollution sources. Interestingly, the authors also highlighted the geographical variability did exist in sensitivity and specificity amongst similar and identical markers. However, this was based only on literature that was available at the time and differences may have resulted from other factors (e.g., local environment, experimental methods).

After *Bacteroides*, another common bacterial assay used for MST targets is *E. coli* (Chern et al., 2011; Warish et al., 2015; Hughes et al., 2017). Gomi et al. (2014) identified thirty-six human-specific genomic regions and chosen four markers against environmental samples alongside several non-human markers. Amongst the human markers, H8 was recommended by the authors due to its high sensitivity and specificity. The authors also noted how *E. coli* may not be suitable for identifying the source pollution in surface water using genetic methods alone, due to its low relative abundance. However, this is unlikely to be an issue in wastewater. More generally, markers targeting *E. coli* may be of particular interest because of their traditional use as faecal indicators.

Numerous other MST assays have been developed and considering the exhaustive range of bacterial, viral and mitochondrial targets is beyond the scope of this review. However, it is worth noting that the application of most methods has generally been local and restricted to several well-studied regions (i.e., the localities of the research groups studying the markers). Their stability and performance in different geographical locations, therefore, is not well understood, although some attempts have been made to rectify this. In their extensive study of 41 MST methods, Boehm et al. tested the sensitivity and specificity of markers targeting human, cow, ruminant, dog, gull, pig, horse, and sheep against corresponding faecal samples and raw sewage (Boehm et al., 2013). Whilst the study provided some useful data for cross-comparing similar markers, it was more or less inconclusive with regards to the geo-stability of different assays. Mayer et al. carried out a 'follow-up' study that investigated five human-associated genetic markers in 29 wastewater treatment plants and 280 faecal samples from 13 different countries (Mayer et al., 2018). The authors found that all markers were consistently abundant in wastewaters across all locations, however, the false positive rate ranged from 5 to 47% when the markers were tested against faecal samples of known origin. Thus, it was concluded that the sensitivity and specificity of genetic faecal markers is regionally or even catchment-specific, which is problematic; i.e., some markers are be more abundant in some locations than in others, therefore not universal. Furthermore, the effects of sunlight and temperature on the stability of the genetic markers varies between organisms (Pachepsky et al., 2014), implying that markers targeting certain organisms might be more or less appropriate in some locations.

2.4.3 Genetic faecal markers and wastewater treatment

Little consideration has been given to the potential use of genetic faecal markers as treatment performance metrics at WWTPs. However, due to the use of sewage as a proxy for human faecal matter when developing new markers, there are many data on the abundance of different markers found in wastewater. Mayer et al. (2016) quantified human-associated *Bacteroidetes* genetic markers in raw and treated wastewater from five larger WWTPs (PE 6600 – 78400) and the final effluent from eight small treatment plants (PE 3 – 130) in Germany and Austria. The small-scale systems were serving either individual households or hotels. The authors found that Bacteroides-based markers were consistently detected in raw and treated wastewater, regardless of system size, treatment type or season. However, it should be noted that the smaller WWTPs were all high-specification package plants that are mandated to meet the stringent discharge standards on all WWTPs in Austria (Langergraber et al., 2018).

Hughes et al. (2017) quantified several bacterial and viral markers, including HF183, in raw and secondary treated wastewater. Interestingly, they found strong, positive correlations between the abundance of HF183 and most other bacterial and viral markers in the raw sewage. However, in secondary treated wastewater, there were no clear correlations except with plant-based pepper mild mottle virus (Spearman correlation = -0.8) and human norovirus (spearman correlation = 0.92). Arguably, it is encouraging that in treated wastewater, HF183 correlated strongly with a moderately common human virus and not with a plant virus, the impact of which on human health is tenuous (Colson et al., 2010).

Beyond these two studies, investigations have focussed on single WWTPs (Srinivasan et al., 2011) or single sample types (Silkie & Nelson, 2009). However, these studies show the potential of genetic markers as treatment performance metrics. By investigating the fate of markers through wastewater treatment processes and comparing the behaviour with conventional, water quality metrics (such as, COD), new knowledge may be gained about the differences between WWTPs of various sizes, operational configurations and technology types.

2.5 Predicting Flow Rates at Wastewater Treatment Plants

The high capital and operational costs associated with wastewater treatment has led to the development of several models to assist with process and design optimisation (Martin & Vanrolleghem, 2014). Platforms such as the Activated Sludge Model (ASM) series and the Benchmark Simulation Model (BSM) series have become common-place amongst wastewater managers and applied extensively by researchers (e.g., Jeppsson et al., 2013; Benedetti et al., 2010; Ostace et al., 2011). However, a limitation of such models is the requirement for large amounts of influent data, including flowrates. This has perhaps limited the use of models, which is compounded by the complexities of on-line monitoring of raw wastewater (Martin & Vanrolleghem, 2014). To overcome this, a range of models have been developed to generate influent data, which typically consists of a time series of the flow and concentrations of typical water quality parameters (e.g., COD, NH_4, TSS). The sources of wastewater vary dramatically between catchments and, particularly at larger scales, the flow and concentration are affected by many parameters (e.g., temperature, soil type, population) (Bott & Parker, 2010).

Martin and Vanrolleghem (2014) point out that the most logical way of generating data is experimentally. The implication is that by collecting enough data to fulfil pre-defined confidence criteria, one can easily infer the 'missing' data. Such an approach could feasibly be applied to predicting wet or dry weather flow profiles; the former relying on substantially more data to retain accuracy. A method proposed by Devisscher et al. (2006) as part of the MAgIC Methodology and applied by others (Gevaert et al., 2009), uses Poission distribution-based inference to generate flow rates within the same time-frame as the measured data. This can be considered reasonable under the assumption that flowrate conditions are predictably variable within a short period of time, which might be the case for larger WWTPs, especially under dry-weather conditions. It provides a useful tool for rapid assessments across a network of sewer catchments. Due to the number of small-scale WWTPs, such an approach might be considered useful, however the requirement for data from which to infer makes it unfeasible; presumably, the more data there is to infer from, the more accurate the inference becomes.

An additional approach to inference is noteworthy and that is the use of machine learning algorithms, such as neural network-based and fuzzy inference techniques.

Such methods have been used, with varying success, to extrapolate influent characteristics for individual WWTPs (Pai et al., 2011, 2009; Cheng et al., 2018; Kusiak et al., 2013). Such methods have been applied to total flow extrapolation, considering flows received under dry and wet weather conditions. Whilst the ever-increasing power of artificial intelligence shows promise for these applications, the number of independent variables required to cover the range of possible situations within each catchment means that these methods might only be relevant for a limited number of catchments – those where all or large datasets are available.

Fourier approximations are another approach used for the prediction of influent characteristics, most commonly under dry-weather conditions (Mannina et al., 2011). The technique has been used to generate hourly-interval dynamic profiles for flow rates or occasionally infer data points that are missing from an existing timeseries (Langergraber et al., 2008). Fourier-based methods have been used for individual and networks of WWTPs (Talebizadeh et al., 2016; Alex et al., 2009).

Phenomenological models such as those developed by Gernaey et al. (2011, 2006, 2005) for the second series BSM (BSM 2), makes use of the observation that human wastewater generation follows a consistent diurnal pattern (Butler, 1993; Friedler et al., 1996) to generate household wastewater flow predictions. They are, thus, particularly useful and effective at predicting flows under dry-weather conditions. It presumes a large peak in flow rate in the morning and evening, corresponding with people going to and returning from work, and flow minima during the night and early afternoon, corresponding with people sleeping and being away from the home during working hours. The Gernaey et al. (2011) model consists of a series of modules, including household flows that exploits the presumed lifestyle patterns plus considerations for weekend and annual variations; industrial flow contributions; seasonal correction factors; rain generation for overland flow; and infiltration via soil through-flow. Total flowrate contributions can then be combined with pollutant parameter modules and used as an input to a series of variable-volume tank models. Such a level of complexity is clearly required for an accurate representation of large sewer catchments. The model is designed to be a compromise on those which offer a deterministic representation of the entire sewer catchment. However, no strategic comparisons appear to have been made between different modelling approaches, across a range of catchments. So, it is unknown what effect such a compromise might have on prediction accuracy.

Finally, deterministic models, including those which are commercially available are some of the most sophisticated ways of generating influent data (Gernaey et al., 2011). Such models are developed for predicting flows under dry and wet-weather conditions. Early attempts highlighted the inherent complexity of this approach (Gustafsson et al., 1993; Hernebring et al., 2002), which typically combine hydrological and hydraulic models to represent a sewer catchment in its entirety. The hydraulic engine underpinning many deterministic sewer models solve the full Saint Venant equations of unsteady flow (Marinaki & Papageorgiou, 2005). Commercially available models, such as Infoworks ICM (formerly Hydroworks; Innovyze, 2019) and MOUSE (now integrated into MIKE URBAN+; DHI, 2019), have been used in many urban drainage studies (Chen et al., 2017; Mahmoodian et al., 2018; Erbe et al., 2002; Fronteau et al., 1997; Koudelak & West, 2008) and their flow prediction capabilities make it possible to predict flow rates received by WWTPs. However, the multifarious nature of catchments, which are dynamic systems, requires a large number of high-resolution input parameters. Such information may not be available for all catchments, particularly those in rural or remote areas.

The lack of flow-rate data is a particular problem for smaller WWTPs, which may be unmonitored due to regulatory conditions, especially in the UK. However, the inference models that have been outlined here are unsuitable for smaller catchments because they either rely on statistical inference from existing data that largely does not yet exist for unmonitored sites or they rely on a deterministic representation of complex physical processes. As such, there is a need for a simple, parsimonious tool which can generate representative flow rate data that can be used to inform the management and potential impact of smaller WWTPs.

2.6 Concluding Remarks

Small WWTPs have an important role to play in ensuring sustainable wastewater management. However, upon considering the current knowledge and tools available to assess such systems, it is clear that there is a gap in understanding. There is a need for new data that allows the comparison of different types and sizes of decentralised treatment systems. There is also a need for new treatment performance metrics which better align with emerging catchment management and public health priorities. Finally, there is a need for a simple influent data generation method which can be applied to a network of small WWTPs to help inform their management and to investigate performance stressors which may be unique to small scales. This thesis presents solutions to meet these needs through the fulfilment of the aims and objectives outlined in Chapter 1.

Chapter 3. A Parsimonious Approach to Predicting Small Wastewater Treatment Plant Reliability

3.1 Introduction

Economies of scale have traditionally been the main argument for centralising wastewater treatment plants (WWTPs) (McCarty et al., 2011). Therefore, research has mainly focussed on large-scale systems, resulting in a wealth of available operating data and firm understanding of their overall performance. By contrast, smaller WWTPs are typically overlooked. This may be because they are often only used where there is no other economically viable option (Larsen et al., 2009); their perceived environmental impact is localised; and/ or they may be exempt from regulatory conditions (DEFRA, 2010). The latter point is of particular importance because regulatory compliance requires monitoring and more effective management. Without mandated monitoring, limited performance data have historically been collected, which now restricts our ability to predict performance and estimate potential discharge impacts. Crites and Tchobanaglous (1998) identified protecting receiving environments as one of the key objectives of decentralised WWTPs and yet the huge data gap is a barrier to assessing the performance of such systems, which is the focus, here.

In the UK, small WWTPs typically serve rural or remote communities and can discharge into sensitive water courses. Although limited data exist, it has long been recognised that the ecological impact of such discharges may be underestimated (Pujol & Lienard, 1989; May et al., 2015). When considering the mechanism by which a waterbody status is determined under the Water Framework Directive (WFD) (EC, 2000), such underestimation is not surprising. The WFD status of a natural surface waterbody for a particular parameter can be calculated from the median value of all sample points within the waterbody (UK Technical Advisory Group on the Water Framework Directive, 2009). Therefore, the water quality at a sample point downstream of a small, poorly managed discharge, may be sufficiently bad to influence the ecological or overall waterbody status.

Despite substantial investment from the UK water industry (and elsewhere), the number of waterbodies in England achieving "good” or better ecological status under WFD, decreased from 26% to 17% between 2009 and 2015 (Environment Agency, 2015). We suspect that inadequate investment in the management, monitoring and construction of small WWTPs may be one reason; greater attention and understanding could be critical to achieving the aims of WFD or other environmental regulatory drivers. However, a lack of evidence on system performance, especially operating stability and net environmental impact, hinders managers and policy decision makers addressing declining ecological quality (Chong et al., 2011). Therefore, here we present new data from twelve rural WWTPs in NE England and contrast them with equivalent data from three larger, regional WWTPs.

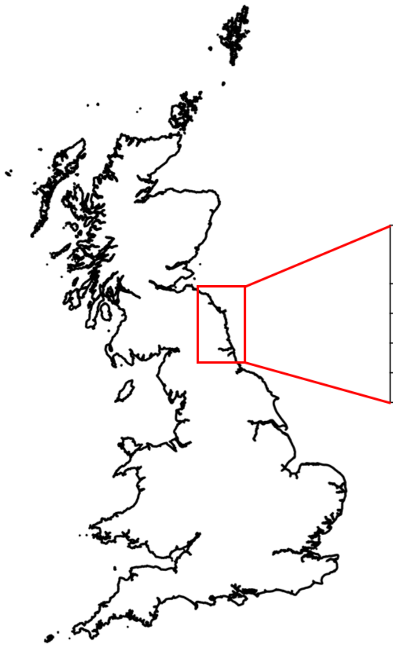
This study specifically sought to assess the effects of size, influent characteristics and technology type on the performance and stability of small WWTPs in the UK. Performance over time (bi-monthly sampling over one year) was assessed by different measures of effluent quality and also removal rates of a range of physical and chemical parameters. Here, a small WWTP is defined as receiving less than 50 m^3 mean flow per day, which is the low-end boundary for the numerical regulation of continuous discharges in England (DEFRA, 2010). This flow roughly equates to a population equivalent (PE) of 250, which is typically used as a proxy measure of size where no flow data are available. Throughout, we use the word ‘limit’ in reference to the legally binding standard placed on a continuous final effluent discharge, which is more often referred to, in the UK, as ‘consent’.

3.2 Methodology

3.2.1 Identification of study sites and experimental design

A list of registered WWTPs in NE England was obtained from the Annual Return made to the Water Services Regulation Authority (OFWAT) in 2016. The database contained information on 412 treatment plants of which 274 (66.5%) have a PE of 250 or less. More specifically, eighty-two are between 50 and 250 PE. For the purposes of this study, the lower limit of investigation was 50 PE. Typically, WWTPs below this size are subject to highly intermittent flows and, within the study region, 55% only are primary treatment systems (i.e. septic tanks) from which obtaining consistent influent samples is difficult.

Four main treatment technologies dominate the list of small WWTPs in NE England, including: rotating biological contactors (RBC), secondary filtration which were trickling filters (SF), activated sludge (AS) and high-performance aerated filters (HiPAF). Two size categories (50-125 PE and 125-250 PE) were used for comparative assessment of size, which created six experimental classes: 50-125 RBC, 125-250 RBC, 50-125 AS, 125-250 HiPAF, 50-125 SF and 125-250 SF, where the number ranges refer to the PE and the letters refer to the technology type. These groupings proportionately represent small WWTPs in the region. A longlist of thirty-six WWTPs was initially generated by stratified random sampling with consideration of proportional allocations to the above six categories. Twelve WWTPs were then chosen for monitoring following site visits to determine accessibility and logistical feasibility. Two plants were chosen in each experimental category (Figure 5; see Appendix A for map of the spatial extent of the study). There were no suitable AS plants between 125 and 250 PE in the study area and no HiPAF plants between 50 and 125 PE. Of the twelve small WWTPs, four had flow monitoring. None of the small sites had discharge limits for either ammonia or phosphorus.



	Nb Total	Nb 50-125 PE	Nb 125-250 PE	Nb Reference
AS	3	2	0	1
SF	6	2	2	2
RBC	4	2	2	0
HiPAF	2	0	2	0

Figure 5 - The number of study sites in each experimental design category and the region of study in NE England. Nb is the number of sites; AS is activated sludge; SF is secondary filtration (trickling filters); RBC is rotating biological contactor; HiPAF is high-performance aerated filter. Contains OS Data © Crown copyright and database right (2019).

Three larger reference WWTPs were chosen to benchmark the performance of the small systems (Figure 5). Specifically, two SF plants and one AS plant were chosen with population equivalents of 7140, 5280 and 9650, respectively. All three reference sites are subject to regulation under the Urban Wastewater Treatment Directive

(UWWTD) (EC, 1991) and have final effluent discharge limits for ammonia. These specific plants were chosen because they predominantly treat domestic wastewater, and the variance of their removal rates of ammonium (NH_4-N) and soluble chemical oxygen demand (sCOD) did not exceed 0.1 between August 2013 and July 2016. This suggests they were comparatively “stable” in terms of routine performance and would be suitable for benchmarking. No tertiary treatment was present at any site and there were no known operational issues. Tertiary treatment is an ambiguous term but typically refers to treatment steps additional to the biological degradation of organic matter and nutrients (Tchobanoglous et al., 2003). Thus, it is commonly used for the removal of specific pollutants prior to discharge to sensitive receiving waters.

The statistical power of the sampling programme was determined *a priori* by two-sided and balanced analysis of variance power calculations using the pwr package in R (Champely, 2018; R Core, 2018). Sensitivity, as Cohen’s D, was set to ‘moderate’ (0.5) as the effect on performance of a treatment plant being in a designated experimental category was unknown at the start of the study (Cohen, 1988). The significance level was set to 0.05; i.e., 95% confidence. Based on logistical feasibility during the field sampling programme, 90 samples were collected across sites for influent and effluent quality and removal rate analyses. Using this sample number, the overall statistical power of the sampling plan was 0.92, implying the sampling regime would produce a dataset suitable for performing statistically significant comparisons across the WWTPs. For each experimental category, 12 influent and 12 effluent samples were collected symmetrically over one year, except for the reference AS plant category, which only had six influent and effluent samples collected over the year. This experimental design provided a statistical power of 0.9 for any inter-category comparisons.

3.2.2 Sampling approach and collection

Manual, time-apportioned, composite samples of raw influent and final effluent were collected at each site, every two months between December 2016 and October 2017. This provided 90 influent and 90 final effluent samples for analysis. Typical peak and daytime base flowrates were determined by calculating the mean time at which these flows occurred at selected WWTPs in the region. Flow data for 2013 – 2016 was obtained from the Monitoring Certification Scheme (MCERTS) flow monitors installed at 25 decentralised WWTPs. Thus, peak flowrate samples were collected between

08:00 and 09:00 and base flowrate samples were collected between 14:30 and 15:30 on the same day of the month for each site. This was important to negate any effect of sample time on pollutant concentrations.

Influent and effluent samples always were collected in 1 L bottles (Nalgene, USA), both at estimated peak and base flows, and samples were combined at the time of collection to create composite influent and effluent samples. The bottles were transferred on ice to Newcastle University and stored at 4 ^{O}C until analysis. On-site measurements of dissolved oxygen (DO) were made using a DO600 meter (Extech, USA). Ambient temperature, wastewater temperature and pH were measured using an EC500 meter (Extech, USA).

3.2.3 Physical and chemical analysis

All analysis was carried out in duplicate within 24 hours of sample collection. The wastewater in each bottle was homogenised by gentle upending. Analysis of total and soluble COD (tCOD; sCOD), ammonium (NH_4-N) and total phosphorus (TP) was carried using colorimetric kits (Merck, Germany) in accordance with Standard Methods for the Examination of Water and Wastewater (APHA, 2009). For analysis of bioavailable fractions of COD and NH_4-N, samples were filtered using a 0.2 μm nylon syringe filter (VWR, UK). Analysis of secondary nutrients was carried out using acid washed plastic to minimise procedural losses. Total suspended solids (TSS) levels were determined by concentrating suspended matter onto a GM6 glass filter membrane (Sartorius, Germany) and incineration at 105°C until consistent weight at five significant figures. Determination of anions of nitrogen (NO_2-N; NO_3-N) was performed by ion chromatography using an ICS-1000 system (Dionex, USA) fitted with an AS40 auto sampler (Thermo Scientific, UK).

3.2.4 Data analysis and statistical observation

All statistical analysis was carried out in R (R Core, 2018) and significance was defined by 95% confidence limits (p < 0.05), unless otherwise stated. Two DO concentration data points were missing in the raw data set and so prior to statistical analysis, were inferred using weighted average K-Nearest Neighbour Imputation with K set to 10, using a pre-scaled dataset. To assess the performance differences between experimental categories, one-way analysis of variance (ANOVA) was used on effluent concentration parameters and removal rates.

3.2.5 Reliability analysis

To assess the stability of the effluent quality from the twelve small WWTPs against the larger benchmark sites, covariance of key parameters was calculated and represented graphically using ggplot2 package (Wickham, 2016). To further assess differences in stability between small treatment plants, reliability analysis (Niku et al., 1979) was carried out using tCOD effluent concentration data. A coefficient of reliability (COR) was derived (Equation 1) from the covariance of the parameter over the sample time-series and the probability of compliance (Equation 2), where *y* is the coefficient of variance; Z_{1-a} is the standardised normal variate obtained from tables generated by Niku (Niku et al., 1979); X_s is the required discharge standard; and m_x is the mean measured effluent concentration for tCOD.

$$COR = \sqrt{(y^2+1)} \times \exp\left[-Z_{1-a}\sqrt{\ln}\,(y^2+1\right] \qquad (1)$$

$$Z_{1-a} = \frac{\ln X_s - (\ln m_x - 0.5\ln(y^2+1))}{\sqrt{\ln}\,(y^2+1)} \qquad (2)$$

The COR was multiplied by the Urban Wastewater Treatment limit to define the “acceptable” final effluent tCOD concentration, which currently is 125 mg-COD/ L for England and Wales (Equation 3), where m_s is the design concentration:

$$m_s = X_s(COR) \qquad (3)$$

The design concentration is defined as the effluent quality that is needed to comply with the required discharge standard at a pre-determined confidence, which here is 99% confidence. Note that this is different to a statistical confidence and derived from lookup tables included by (Niku et al., 1979).

3.2.6 Statistical modelling of treatment plant reliability

The probability of tCOD effluent concentrations exceeding the calculated design concentration at each small-scale WWTP was predicted using random forest (RF). RF is a powerful machine learning classifier which has key advantages including being robust to outliers and dataset noise, and the ability to identify parameter importance (Brieman, 2001; Ho, 1995). The approach to modelling is a supervised learning algorithm that relies on the construction of an ensemble of decision trees, which is particularly useful for correcting overfitting commonly observed in decision trees (Hastie et al., 2008). Thus, the approach is particularly useful for this application where the ratio of sample numbers to unique sample sites, which could ordinarily result in model overfitting. Modelling was done using the caret package (Kuhn, 2018) in R with a 70 / 30 randomly determined train/ test data split, which was also chosen because of the ratio of sample numbers to the number of unique sample sites. Cross-validation was carried out ten times by comparing the area under the Receiver Operating Characteristics (ROC) curve as the metric for model performance (Metz, 1978). The optimised model was built using fifty-nine trees which was identified as the point at which the minimum error rate occurred in model training.

Cross-correlations between predictor variables were determined using Spearman's rank correlations. The predictor values used were influent concentrations of tCOD, TSS, NH_4-N and DO, influent pH and temperature, atmospheric temperature, season, PE, treatment technology type and the number of times that the site was visited by an operator each week. Concentrations of TSS and tCOD in the influent samples had an r^2 of greater than 0.75, therefore TSS was removed from the model dataset to reduce the chance of false positive predictions. The importance of each predictor variable was calculated by comparing the cross-validated mean standard error (MSE) of the model performance with the performance when withholding each predictor in turn. The resulting differences were averaged and normalised by the standard error, and the parameter causing the greatest difference in normalised MSE was determined to be the most important. This approach was chosen because of the non-linear nature of the relative trends among key parameters, thus rendering traditional linear methods of predictor significance inappropriate. Data was scaled and centred prior to modelling.

3.3 Results and Discussion

3.3.1 Analysis of experimental categories

A summary of effluent concentrations and removal rates for the smaller and larger WWTPs is presented in Table 1. Final effluent sCOD concentrations for the twelve smaller WWTPs ranged from 21 mg/L to 317.5 mg/L with a mean value of 64.1 mg/L. The range for tCOD concentrations at smaller systems was 22.0 to 727 mg/L, which is strongly correlated with TSS. The mean effluent tCOD was 114.6 mg/L. By contrast, at the larger benchmark plants, maximum effluent COD levels were an order of magnitude lower than smaller plants with mean effluent concentrations for both sCOD and tCOD, which are 64.0 mg/L and 77.5 mg/L, respectively. Effluent NH_4-N concentrations at the smaller WWTPs ranged from 1.75 mg/L to 49.2 mg/L with a mean value of 16.5 mg/L. Whereas, NH_4-N concentrations in the final effluent of the larger WWTPs was on average 2.2 mg/L (never exceeding 5.2 mg/L), which meets regulatory compliance levels.

(a) Effluent concentration

	Smaller WWTPs (n=72)				Larger WWTPs (n=18)			
Parameter	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**
sCOD (mg/L)	21	317.5	64.1	44.8	17	64	35.6	14.8
tCOD (mg/L)	22.5	727	114.6	108.2	28.5	77.5	48.3	15.3
TSS (mg/L)	2.3	161	31.6	35.3	5.5	19.3	12.1	4.1
TP (mg/L)	1	11.6	4.9	2.5	1	5.1	3.6	1
NH_4-N (mg/L)	1.75	49.2	16.5	12.1	1	5.2	2.2	0.8
NO_3-N (mg/L)	LOD	129.5	13.4	21.7	LOD	104.1	22.8	27.5
pH	5.73	8.03	7.2	0.4	5.9	7.7	7.2	0.4
DO (mg/L)	1.1	7.7	3.2	1.2	1.6	6.4	4.1	1.5
Ambient temp (^oC)	-1.4	24.3	11.5	5.5	4	20.9	13	4.6
WW temp (^oC)	4	19.1	12.2	4.6	6	17.3	12.6	3.4

(b) Removal rates

	Smaller WWTPs (n=72)				Larger WWTPs (n=18)			
Parameter	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**
sCOD (%)	-18.4	95.1	67.3	20.4	33.5	94.3	73.2	17.6
tCOD (%)	-13.1	98.5	78	20.6	74.1	96.6	88.5	7.03
TSS (%)	-13.6	98	80	33.9	80.3	97.6	91.7	4.6
NH_4-N (%)	-15.4	95.4	55.5	30.4	81.6	96.6	92.9	3.7

Table 1 - Statistical observations of final effluent and removal rate parameters for smaller and larger reference WWTPs. LOD = limit of detection; WW = wastewater.

The effluent quality for the smaller WWTPs also was much more variable than the larger plants for all parameters, except pH and DO. The largest observed standard deviation (SD) among effluent parameters was for tCOD at both the larger and smaller WWTPs. For larger plants, this is likely because of a relatively ‘generous’ limit of 125 mg/L imposed under the UWWTD; i.e., most treatment plants produce effluent concentrations far below this level, which allows less stringent process control. In contrast, no tCOD regulation typically exists on discharge concentrations for the smaller WWTPs, therefore they are not routinely controlled. This is evident in the measured highest effluent concentration of 727 mg/L, which is six times higher than the mean. The lowest SD was observed in pH and DO effluent values.

In terms of removal rates, the parameter with highest mean rate of removal at smaller WWTPs is TSS (80.0%), whereas mean removal rates are highest for NH_4-N at the larger WWTPs (92.9%). The SD of removal rates across larger plants was lowest for NH_4-N which, again, is probably a result of tight discharge regulations. The lowest SD amongst removal rates at smaller WWTPs was for sCOD, but this was still > 20 and suggests a high level of variance in effluent quality. In fact, one small WWTP had effluent quality poorer than influent quality. The lowest SD at the larger WWTPs was for NH_4-N (3.7).

There is a significant difference between the mean effluent values of the design categories across all parameters except NO_3-N at 95% confidence (ANOVA, $4e^{-10} < p < 3.9e^{-3}$; $p = 0.06$). The similarity between NO_3-N effluent values may be because most small WWTPs serve rural communities that include farms that might lead to an increased load of NO_3-N entering the wastewater collection system, which would not be removed and so appear, similarly, in each effluent discharge. However, without being able to determine load fluxes or specific process mechanisms, it is not possible to confirm this speculation. Other than NO_3-N, the least confidence in significance was between pH of final effluent samples, which is not surprising when considering SD of values for both small and larger plants (Table 1). For removal rates, there also is a significant difference between the removal rates at the different WWTP sizes and technologies, across all parameters (ANOVA, $2.5e^{-9} < p < 2.5e^{-4}$).

3.3.2 Covariance of effluent parameters

Covariance data on final effluent parameters from the twelve small WWTPs is summarised in Figure 6. The correlation between the mean effluent concentration and the SD is strongest for tCOD ($r^2 = 0.93$). This demonstrates a strong relationship between the treatment performance and operational stability across treatment systems. A similarly strong trend was seen for sCOD and TSS ($r^2 = 0.75$ for both).

Correlations for NH_4-N also are strong ($r^2 = 0.84$), which is surprising because none of the small WWTPs had a discharge limit for NH_4-N at the time of the study. This is interesting because the smaller WWTPs are unlikely to have been designed for or operated in order to achieve nitrification, and yet there are evidently some treatment systems consistently achieving some nitrification. This suggests that observed trends of covariance probably are a ‘natural’ phenomenon rather than a result of operational practices or engineered design. In other words, conditions promoting nitrification have occurred by ‘chance’ and have developed to be relatively stable through time.

In terms of TP, while there is a significant difference in removal rates between the large and small WWTPs (ANOVA, $p < 0.05$), covariance trends between performance and stability are relatively weak ($r^2 = 0.45$). None of the monitored WWTPs have phosphorus removal technologies and it is much less likely that TP removal, especially by enhanced biological removal, will occur by chance than, for example, nitrification might. The three larger treatment systems are clustered to the lower left-hand corner of the plot (i.e., higher quality effluent and greater stability) for all parameters except for TP. After this, the next most obvious observation on performance versus stability covariance trends is differences among technology types. The package plants tend to discharge higher quality effluent on average and do so more consistently. For example, SD of NH_4-N ranged between about 3 and 8 mg/L for RBC and HiPAF treatment types (Figure 6e). It was, however, not possible from this covariance analysis to exactly determine the role treatment type (or any other factor) played in the stability of effluent quality.

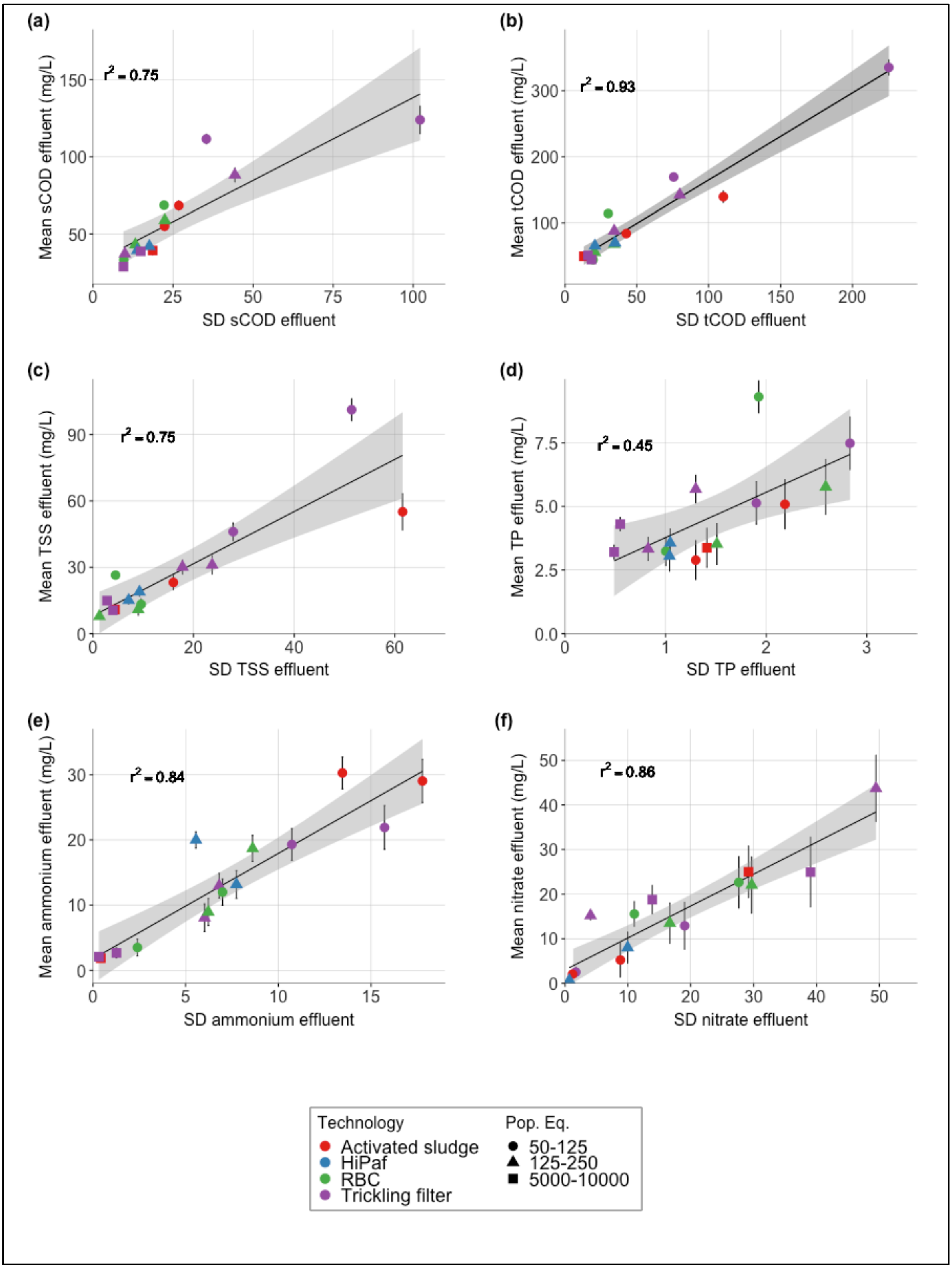


Figure 6 - Covariance plots for final effluent values by experimental category as population equivalents and technology type. Colours identify treatment technology type and shape identifies the population equivalence. Error bars show standard error. Shading shows confidence in the linear regression smoothing at the 99th percentile. All correlations (reported as r^2), are significant ($p < 0.01$). Plot (a) is soluble COD; (b) is total COD; (c) is total suspended solids; (d) is total phosphorus; (e) is ammonium and (f) is nitrate.

3.3.3 Reliability small treatment systems

Design concentrations for tCOD for each small WWTP are summarised in Figure 7, grouped by the WWTP size and technology type. The lowest effluent concentration required to maintain compliance with UWWTD tCOD discharge standards at 99% confidence was 63.7 mg/L. Given this criterion, it is not surprising that one of the 50-to-125 PE trickling filters had the highest mean tCOD effluent concentration, well beyond discharge standards (727 mg/L). The highest design concentration was 78.2 mg/L, which was calculated for the RBC with a PE of between 50 and 125.

Whilst the range of design concentrations is relatively small (14.5), there is a clear inverse relationship between the measured and design concentrations (Figure 7). However, two WWTPs that have mean effluent concentrations of > 125 mg/L had design concentrations higher than three of the treatment systems with mean concentrations > 125 mg/L. This confirms that calculations driven by covariance and probability analysis are not simply the average of measured values or numerical distance from the mean (i.e., SD). Means and SDs are both useful at times, but ultimately, are limited measures of performance because of the underlying assumptions upon which their implications depend. Specifically, the assumption of a Gaussian or additive normal distribution (Limpert et al., 2001), which may not summarise the characteristics of every parameter of interest. Therefore, other methods are needed to better understand performance trends, which may allow a deeper insight into risks of WWTP compliance failure, ideally also aimed at ecological improvement in catchments. Whilst we do not endorse neglecting sites that appear to provide stable performance naturally, increased awareness of a WWTP's reliability means that operational practices and allocation of resources can be optimised.

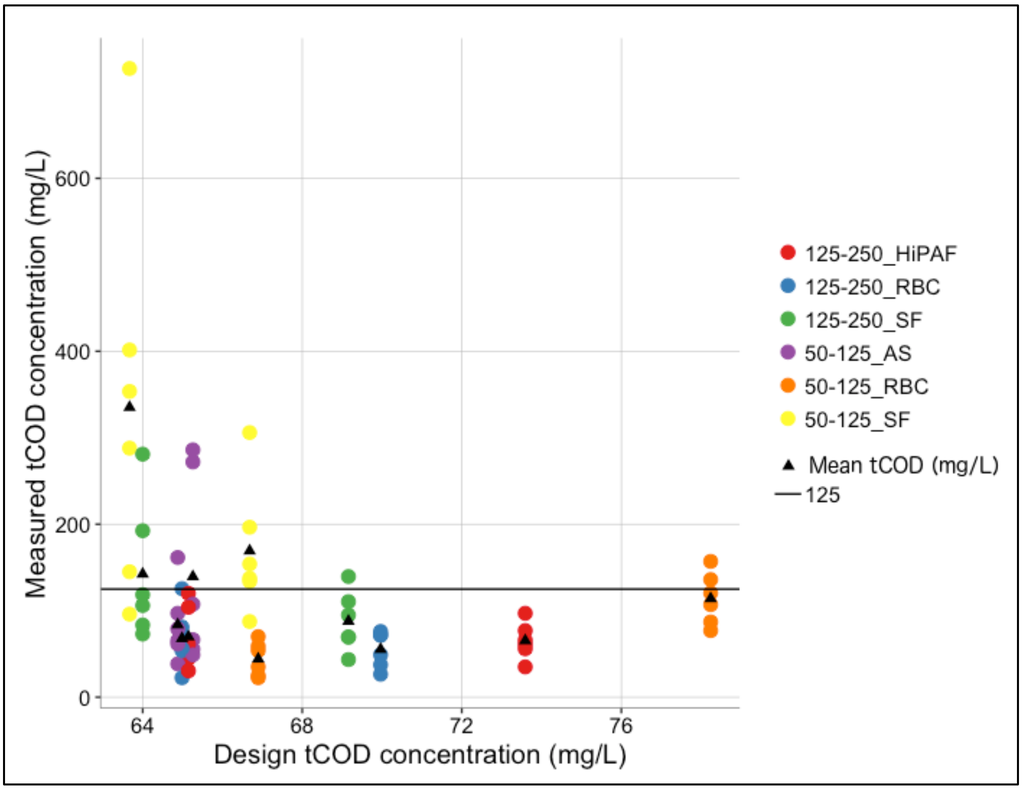


Figure 7 - Mean design and mean measured final effluent concentrations of tCOD for each study site and each experimental design category for small WWTP only. Black line denotes the UWWTD regulatory discharge limit of 125 mg/L COD. Black triangles denote mean measured effluent tCOD values for each site. n=90.

Experimental groups with the most similar design concentrations, and therefore, the most similar effluent quality (measured as tCOD concentration, only), are small AS WWTPs with a PE between 50 and 125 (50-125_SAS). However, considering the position of these two systems in the covariance plots (Figure 6), it is apparent the observation is also relevant for other treatment performance parameters.

3.3.4 Prediction of small works reliability

Whilst it is useful to observe the evident similarity of effluent quality that was discharged from small AS plants, it is perhaps more important to understand what drives or influences such trends. The adage, 'no two WWTPs are the same' may be true, but there also may be enough similarity between the performance of different systems to identify dominant predictors. Thus, we applied a simple machine learning algorithm to predict the reliability of the small WWTPs assessed in this study, which determines the likelihood of tCOD effluent concentrations exceeding site-specific design concentrations (Figure 7).

The optimised random forest model was used to predict the likelihood of the effluent concentration being above the design concentration with an accuracy of 64.2% and, therefore, a mean standard error of 0.358. This model was chosen after comparison with the performance of a gradient boosting machine and a generalise linear model (see Appendix B for further details on the performance of different models). The random forest model correctly predicted the effluent tCOD concentration exceeding the design concentration for 71.4% of the samples. In contrast, the model correctly predicted the effluent tCOD concentration not exceeding the design concentration for 57.1% of the samples (Table 2). This suggests the model is conservative, which may appeal to risk managers responsible for prioritising asset investment against regulatory compliance or environmental targets. Such an approach might be useful to forecast the performance reliability of multiple small WWTPs, simultaneously. The implication of the data is that there may be enough similarity between different sites to establish underlying trends and drivers of performance.

		Reference	
		Actual > Design	**Actual < Design**
Prediction	**Actual > Design**	71.40%	42.90%
	Actual < Design	28.60%	57.10%

Table 2 - Confusion matrix for random forest model prediction showing the percentage of correctly predicted tCOD concentration values.

Considering the performance of the model for each of the six experimental categories for the small WWTPs, it is clear that the reliability of the package plants (especially, RBCs) is harder to predict that the more traditional technologies (Table 3). For example, the model correctly predicts the likelihood of the effluent concentration falling below the design concentration for all samples collected at trickling filter sites. This is likely because the stability of effluent quality discharged from the RBCs is generally higher than other plants which makes the difference between the measured effluent concentration and the design concentration is small. It should be noted that with two WWTPs in each experimental category, it is difficult to attribute the model performance to characteristics inherent to that particular technology type. This is particularly pertinent when considering the model performance for small AS treatment plants; for example, 50% accuracy could be attributed to the correct prediction of samples collected at one WWTP and not the other. However, the similarity of the design concentrations (Figure 7), suggests this may not be case, here. Furthermore, there is a clear distinction between the accuracy of the model for some experimental categories, over others.

WWTP Category	% Correct Predictions
125-250_HiPAF	67
125-250_RBC	17
125-250_SF	100
50-125_AS	50
50-125_RBC	67
50-125_SF	100

Table 3 - Random Forest model accuracy by experimental category.

The relative value of different model predictors is shown in Figure 8, which shows that influent wastewater characteristics and PE are most important. All samples were used to determine the most important predictors, rather than only samples collected at WWTPs for which the model performed particularly well because the approach presented, here, is designed for relevance to a system of small WWTPs, rather than only those that are ‘easy’ to predict and, potentially, therefore, more manageable. Interestingly, the size of a treatment system appears to be more important to consistency in effluent quality than the treatment technology itself. This is supported, at least in part, by the variance observed between treatment plants within the same experimental category and differences among categories (Figures 6 and 7). Further, the smallest WWTPs (50-125 PE) appear to be consistently less stable (i.e. greater variability in effluent quality) than the sites with a PE between 125 and 250. It may not be appropriate to categorise all WWTPs according to these PE bands, but the model outputs combined with the analysis of the experimental categories suggest these groupings may be sufficient and useful for assessing the influence of different parameters on treatment performance.

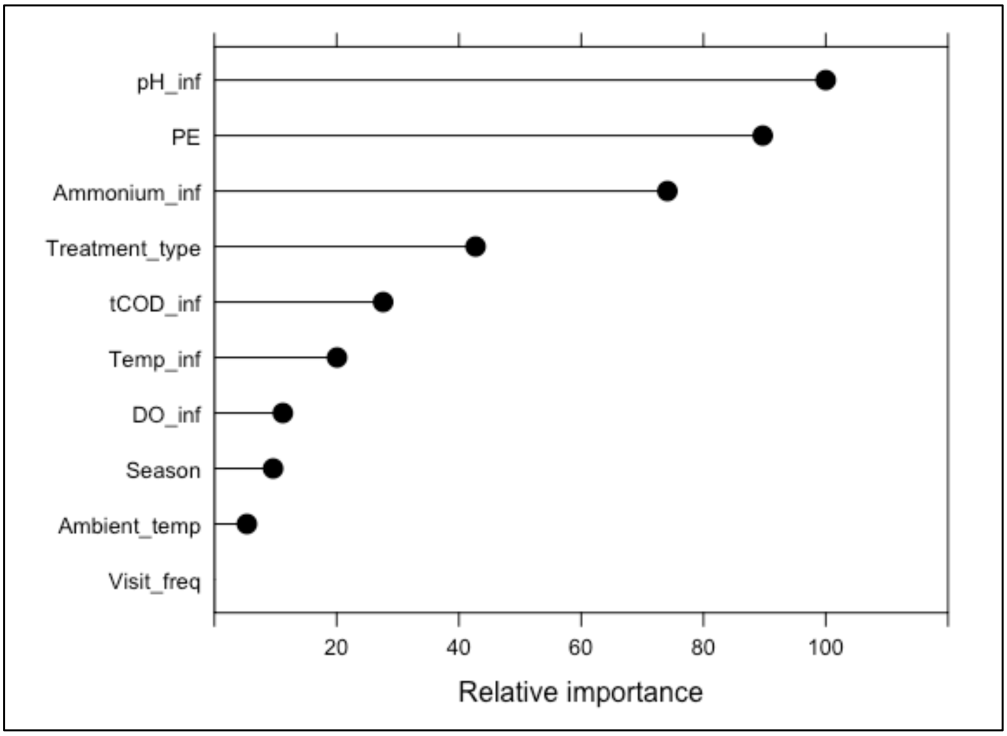


Figure 8 - Relative importance of predictors as determined by random forest. 'pH_inf' is the pH of the influent wastewater; 'Ammonia_inf' is the concentration of ammonia in the influent wastewater; 'PE' is the population equivalence; 'Treatment_type' is the treatment plant technology; 'Temp_inf' is the temperature of the influent wastewater; 'tCOD_inf' is the concentration (mg/L) of tCOD in the influent wastewater; 'Visit_freq' is the number of times an operator visits the site per week; 'DO_inf' is the concentration of dissolved oxygen in the influent wastewater; 'Season' is UK season; 'Ambient_temp' is the atmospheric temperature at the time of sample collection.

In contrast to system size and influent characteristics, most other predictors had little relative importance in predicting effluent stability (< 60, Figure 8). The significant difference (unpaired t-test, $p < 0.05$) between wastewater and ambient air temperatures implies a buffering effect against the latter. This explains why seasonal changes are relatively unimportant as a predictor of resilience. However, whilst the temperature of the liquid influent was somewhat important, it does not appear to be a dominant predictor in this model. Interestingly, the DO concentration of the influent also has little relative importance. This is likely because the effects of aeration capacity or hydraulic retention time, which are not considered here, both influence performance regardless of the influent DO concentration.

The final parameter of note relative to system performance is the frequency of visits to sites by operators. This parameter is included here as a predictor of the effect of operational practice. In the UK and elsewhere, the frequency in which small WWTPs are visited by operators can vary from several times per week to once every couple of months. The frequency of operator visits appears relatively unimportant and a poor predictor of WWTP stability (Figure 8). This might be because the actual activity during each site visit can vary, both between sites and through time. Activities might range from checking pumps and plumbing, assessing controls, cleaning lines and other incidental activities. However, implicitly, this suggests the original design and sizing of the processes are more important to treatment performance. This seems to be especially true of smaller WWTPs that do not appear to be improved by simply increasing operational maintenance.

3.3.5 Model simplification

In an attempt to simplify the predictive model, all input parameters with a relative importance below 75 (Figure 8) were removed. This meant the independent variables were pH of the influent, NH_4-N concentration of the influent and the PE. The presence of influent pH and NH_4-N concentration in this list may be because they are acting as indicator metrics for the overall wastewater ‘strength’, rather than because the pH or NH_4-N themselves control the reliability of tCOD effluent concentration. RF classification using the same input conditions and training dataset as previously described, generated an accuracy of 66.1%, which is an increase of approximately 2% compared to modelling with all parameters. Whilst such a marginal improvement might be attributed to chance, it is encouraging that the prediction of small WWTP reliability can be condensed to just three parameters without any loss of accuracy. This is important because it limits the data requirements at small sites and still allows wastewater managers to predict the likelihood of these systems becoming unreliable.

3.4 Conclusions

Limited understanding of small WWTPs is driven largely by a lack of available operational performance and impact data. Here we show the stability and effluent quality of smaller systems is significantly poorer than their larger counterparts. However, the influence of size extends beyond what has been previously recognised, especially how system size relates to consistent compliance with possible limits. Specifically, the smallest WWTP (50-125 PE) appeared less stable than the slightly larger WWTPs (125-250 PE), across all technology types. These trends also reflected in the reliability of the different systems. A simple model showed that the reliability of the effluent quality discharged from small WWTPs can be predicted using just three parameters to a reasonable degree of accuracy.

More generally, the work also shows how simple mathematical techniques can be used to provide insight into the performance and reliability of smaller WWTPs and might be used to improve operational efficiency. Such analysis can inform a more strategic approach to managing effluent releases in rural and remote catchments, particularly to achieve regulatory compliance, reduce environmental impact, or prioritise operational and capital investment. There is a growing recognition of the benefits of decentralised wastewater infrastructure and the methods applied, here, could lead wastewater and asset managers to realise the potential of such systems, including the role they can play in improving ecological ambitions.

Chapter 4. Use of Genetic Faecal Markers as Treatment Performance Metrics for Small Wastewater Treatment Plants

4.1 Introduction

The design and regulation of WWTPs is almost exclusively for the benefit of environmental protection. Yet in many countries, the potable use of untreated water means that wastewater discharges also can pose a health threat; i.e., up to 4% of deaths globally are attributable to poor sanitation and hygiene (Troeger et al., 2017). Several studies have quantified bacterial and viral genetic markers in and out of WWTPs (e.g. Mayer et al., 2015; Brown et al., 2015) but few have considered the use of such parameters directly as treatment performance metrics. This may be because risks to human health from exposure to wastewater discharges are often difficult to define (Huijbers et al., 2015) or simply because such markers are not regulated. However, with recent and rapid advancements in molecular methods, their value and use must be considered.

As background, the advent of MST has resulted in the development of a large suite of genetic faecal assays (Harwood et al., 2014). Such techniques can be used to attribute faecal pollution to specific sources, which allows public health managers to better quantify and mitigate risks to human health. In contrast, traditional measures of sewage pollution rely on the quantification of culturable organisms such as *E. coli* or enterococci. These methods are simple, low cost and therefore, widely used. However, unlike genetic markers, culture-based methods do not differentiate between sources of pollution. This is important since current microbiological water quality standards are based on health-risk, which differs depending on the source of pollution (Seurinck, Verstraete, et al., 2005). In contrast, genetic faecal markers can be more specific (e.g., human versus non-human sources), allowing more accurate allocation and relative quantification of different risks. As such, they could also help to quantify relative source-specific faecal loads entering a watercourse, guiding wastewater and water quality management decisions.

There is an abundance of data on the suitability of markers for tracking human-derived pollution. In the development of MST assays, domestic wastewater is commonly used to test the sensitivity and specificity to human-associated markers. Furthermore, specific markers have been quantified in different waste streams (Srinivasan et al., 2011; Mayer et al., 2018, 2016). However, such testing has more been to determine global or regional variance of human reference samples. Such methods have not extensively been used to carry out detailed assessments of treatment performance within a strategically designed study. More specifically, there have been no attempts to use genetic faecal markers as a metric of treatment performance in terms of final effluent quality or gene removal rates.

Here, we assessed selected genetic faecal markers within the context of possible faecal releases from small WWTPs. Such systems are of particular interest because their performance, stability and environmental impact are traditionally not well understood. However, there is growing recognition of the benefits of decentralised treatment over centralised infrastructure, based on whole-life economic costs and more accurate sizing for local needs (Roefs et al., 2017; Wang, 2014). The latter is particularly pertinent in developing countries where population growth rates far exceed the rate of sewage infrastructure investment (Maurer et al., 2005; Graham et al., 2019). In such contexts, the human exposure risk to sewage polluted waters is also often greater and more overt. Furthermore, the potential for localised wastewater reuse has been highlighted in recent times and is particularly pertinent with growing, international awareness of water scarcity. Therefore, it is of global relevance that small WWTPs are better understood, particularly potential risks their discharges pose to human health. The aim of this study was to assess how the use of genetic faecal markers to might inform this need and also, provide new insight into WWTP treatment performance by providing an alternative, perhaps more pertinent, treatment performance indicator.

4.2 Materials and Methods

4.2.1 Experimental design and sample collection

Influent and final effluent wastewater samples were collected from fifteen WWTPs in NE England and analysed for physical and chemical performance parameters as previously described (Chapter 3). Briefly, six categories of small WWTP (defined as 250 or less PE) were identified by random stratified sampling of a list of all local plants and two were chosen for each category, totalling twelve small WWTPs. Experimental categories consisted of two brackets of population equivalence: 50-125 and 125-250, and four technologies: activated sludge (AS), secondary filtration (trickling filter, SF), rotating biological contactor (RBC) and high-performance aerated filter (HiPAF). Three larger WWTPs, two trickling filters and one activated sludge plant, were chosen to provide a performance reference against which to contrast the smaller WWTPs. Thus, the experimental categories were: 50-125_SF, 125-250_SF, 50-125_AS, 125-250_HiPAF, 50-125_RBC, 125-250_RBC, 5000-10000_SF, 5000-10000_AS.

Analysis of soluble chemical oxygen demand (sCOD), total chemical oxygen demand (tCOD), ammonium (NH_4-N), total suspended solids (TSS) and anions of nitrogen (NO_2-N and NO_3-N) was carried out on ninety influent and ninety effluent samples collected between December 2016 and October 2017. Detailed results of these parameters were presented in Chapter 3.

4.2.2 Selection of faecal markers

The faecal markers used in this study are shown in Table 4. Human-associated and non-specific faecal markers targeting *E. coli* and *Bacteroides* were chosen due to their high reported sensitivity and specificity (Warish et al., 2015; Ahmed et al., 2016; Harwood et al., 2014). For human-associated *Bacteroides*, HF183/BacR287 (Green et al., 2014) and HumM2 (Shanks et al., 2009) were chosen and for human-associated *E. coli,* H8 (Gomi et al., 2014) was used. AllBac (Layton et al., 2006) and RodA (Chern et al., 2011) also were used to quantify the total number of *Bacteroides* and *E. coli* genes, respectively, and also for quality control to rule out the possibility of PCR inhibition in sample dilutions. *Bacteroides* is of particular interest because of its high abundance in the human gut (Seurinck, Verstraete, et al., 2005) and HF183 is now widely used in MST studies (e.g., Chase et al., 2012; Wanjugi et al., 2016; Cao et al., 2018). The US EPA developed HumM2 marker was chosen because of its relatively high performance when compared to other similar markers (Ahmed et al., 2016). In contrast, the *E. coli* markers were chosen for comparison because of the long-standing use of culturable *E. coli* as a faecal indicator.

Assay	Target	Sequences (5' to 3')	Ref.
RodA	Total E. coli	F: GCAAACCACCTTTGGTCG R: CTGTGGGTGTGGATTGACAT	Chern et al., 2011
H8	Human E. coli	F: ACAGTCAGCGAGATTCTTC R: GAACGTCAGCACCACCAA	Gomi et al., 2014
AllBac	Total Bacteroides	F: GAGAGGAAGGTCCCCCAG R: CGCTACTTGGCTGGTTCAG	Layton et al., 2006
HF183	Human Bacteroides	F: ATCATGAGTTCACATGTCCG R: CTTCCTCTCAGAACCCCTATCC	Green et al., 2014
HumM2	Human Bacteroides	F: CGTCAGGTTTGTTTCGGTATTG R: TCATCACGTAACTTATTTATATGCATTAGC	Shanks et al., 2009

Table 4 - Genetic faecal markers used in this study. F = forward sequence, R = reverse sequence.

4.2.3 Quantification of faecal markers

Wastewater samples were returned to the laboratory on ice and within three hours of collection biomass was concentrated onto cellulose nitrate membrane filters (0.22 μm Sartorius, Germany) from 20-50 mL of influent or 50-250 mL of effluent wastewater. Filters were frozen at -20 ^{O}C until bulk extractions were performed. For DNA extraction, cells were lysed for 40 s using a FastPrep R-24 rybolyser (MP Biomedicals Inc., USA) with the speed set to 6 m/s. Extractions were carried out using Spin kit for Soil (MP Biomedicals Inc., USA) according to the manufacturer's protocol.

Each well in 96 well plates were loaded with a master mix consisting of 5 uL SsoFast Evergreen Supermix (Bio-rad, USA), 500 nm of primers, 2 uL of DNAase-free water and 2 uL of template DNA, providing a total reaction volume of 10 uL. qPCR analyses of each influent wastewater DNA sample was run at 10^{-1} and 10^{-2} dilutions, whereas final effluent DNA samples were run at 10^{-1} and 10^{0} dilutions using a CFX96 qPCR machine (Bio-Rad, USA) according to the SsoFast Evergreen Supermix manufacturer’s protocol with annealing temperatures set to 60 °C for all primers, which is consistent with the referenced literature (Table 4). The dilution that resulted in the lowest, mean quantification cycle value was used for subsequent analysis. For each qPCR run, triplicate no template controls (NTC; i.e., DNA replaced with DNAase-free water) also were analysed to assess possible contamination or unexpected amplification. NTC results were consistently negative.

Quantification standards were developed as linear sequences amplified from DNA extracted from target organisms and cleaned using the MinElute PCR purification kit (Qiagen, Netherlands). Linear sequences were chosen to avoid overestimation that can be observed in supercoiled plasmid standards (Hou et al., 2010), which was important because more than one assay was used to target the same organism (human-associated *Bacteroides*). qPCR efficiencies always were between 89% and 107%, and the calibration curve R^2 was at least 0.99 for all runs, which exceeds the Minimum Information for publication of Quantitative real-time PCR Experiments guidelines (MIQE; Bustin et al., 2009). Based on experience and retaining simplicity, the limit of detection for each marker was defined as 10 gene copies per reaction, which also is consistent with previous MST studies (McQuaig et al., 2009; Ahmed et al., 2008).

4.2.4 Statistical analysis

All statistical analysis and data visualisation were carried out using R (R Core, 2018) and associated packages. Significance is defined at the 95th percentile ($p < 0.05$), unless otherwise stated. The effect of experimental category – system size and treatment technology – on the removal rate of each faecal marker was tested by one-way Analysis of Variance (ANOVA). Data was scaled and centred prior to all analysis. One outlying effluent data point was removed from the winter and summer datasets used for clustering analysis, which was identified by assessing its relative deviation from the x-y distribution on the quantile-quantile normal plots for each marker. Associated chemical data from the same data point corroborated this outlying effect (e.g., see sCOD concentration in Table 1).

To test the suitability of genetic faecal markers for assessing WWTP performance, hierarchical and partitioning clustering algorithms were combined with principle component analysis. K-medians clustering was used to identify the markers that best describe the variance between the effluent qualities observed at treatment plants. A partitioning approach was chosen because it is computationally efficient and because it describes the distance between the effluent data points and the centre of the respective cluster. K-medians was chosen specifically because it is less sensitive to outliers than similar approaches, such as k-means. The appropriate number of clusters was chosen by plotting the within-group sum of squares for each partition and identifying the point at which the plot 'levels'; i.e. when the number of clusters no longer influence the within-group sum of squares (Hothorn & Everitt, 2014).

Ward clustering was used to test seasonal effects on effluent quality for all parameters across each experimental category and also to test the similarity of effluent concentrations between experimental categories. Ward clustering and the generation of heat maps for visualisation was done using the made4 package (Culhane et al., 2005) in R. Ward clustering was chosen due to the expected homogeneity of effluent qualities measured at WWTPs in some experimental categories, for example 50-125_SF and 125-250_SF. Ward clustering aims to find compact, 'spherical' clusters (Ward, 1963), whereas other methods (e.g., complete or single linkage methods) adopt less constrained approaches, such as 'friends of friends' which would likely infer unrealistic similarities.

Clustering analysis was carried out on samples collected during the summer and winter, which was used to investigate seasonal effects on genetic marker abundances. For this study, summer was defined as samples collected at the start and end of the UK meteorological summertime (June and August), whereas winter includes samples collected at the start and finish of the UK meteorological wintertime (December and February).

4.3 Results & Discussion

4.3.1 Characterisation of WWTPs and chemical wastewater quality

The abundance of five genetic faecal markers was quantified in the influent and final effluent of fifteen WWTPs in NE England - twelve smaller WWTPs with design capacities of between 50 and 250 PE, and three larger, reference WWTPs with design capacities between 5000 and 10000 PE. For reference, physical and chemical performance characteristics for all WWTPs are summarised in Table 1.

4.3.2 Quantification of faecal markers in WWTP influent and effluent

All markers were detected in 100% of samples (n = 120). Concentrations of each marker in the influent and final effluent samples from the small WWTPs are shown in Figure 9a and for the larger WWTPs in Figure 9b. Median concentrations of the human associated *Bacteroides* markers in influent samples were very similar for both the small and larger WWTPs. Influent concentrations in the small WWTPs were $\log_{10}$ 6.34 and $\log_{10}$ 6.6 of HF183 and HumM2, respectively, whereas $\log_{10}$ 6.23 and $\log_{10}$ 6.64 were detected in larger plant influents. However, a significant difference was observed between median effluent abundances of HF183 and HumM2 in the small versus larger WWTPs (Welch's two-sample t-test; $p = 0.003$ for HumM2; $p = 0.02$ for HF183). The median concentration of total *Bacteroides* (i.e., AllBac) was two to three orders of magnitude higher than HF183 and HumM2 in both the effluent and influent wastewater for both the small and larger WWTPs, which is consistent with previous findings (Mayer et al., 2015).

For human associated *E. coli*, H8, median abundances in the influent and effluent samples from the small WWTPs were $\log_{10}$ 5.42 and $\log_{10}$ 3.85, respectively. This is one to two orders of magnitude lower than the human associated *Bacteroides* markers and two orders of magnitude lower than the mean abundances of total *E. coli* (i.e., RodA). The difference between the effluent qualities at the small and the larger WWTPs also was observed in the difference in *E. coli* marker concentrations. There was a significant difference between the final effluent abundances of H8 and RodA at the small WWTPs verses the larger WWTPs (Welch's two-sample t-test, p = 0.001 - 0.01).

To test the effect of experimental category (i.e., treatment technology type and system size) on faecal marker abundances, ANOVA models were applied to influent, final effluent, and pooled (influent and final effluent combined) samples. Pooling was possible because of the homogenous distribution of samples across all markers and sample types. There was no significant difference between the abundance of any markers when only considering the influent or the effluent samples across the WWTP categories, suggesting the chosen experimental categories cannot reliably describe the variance in influent or effluent faecal marker abundances in the small WWTPs. In other words, factors other than treatment technology type and PE appear to drive the influent and effluent faecal marker abundances.

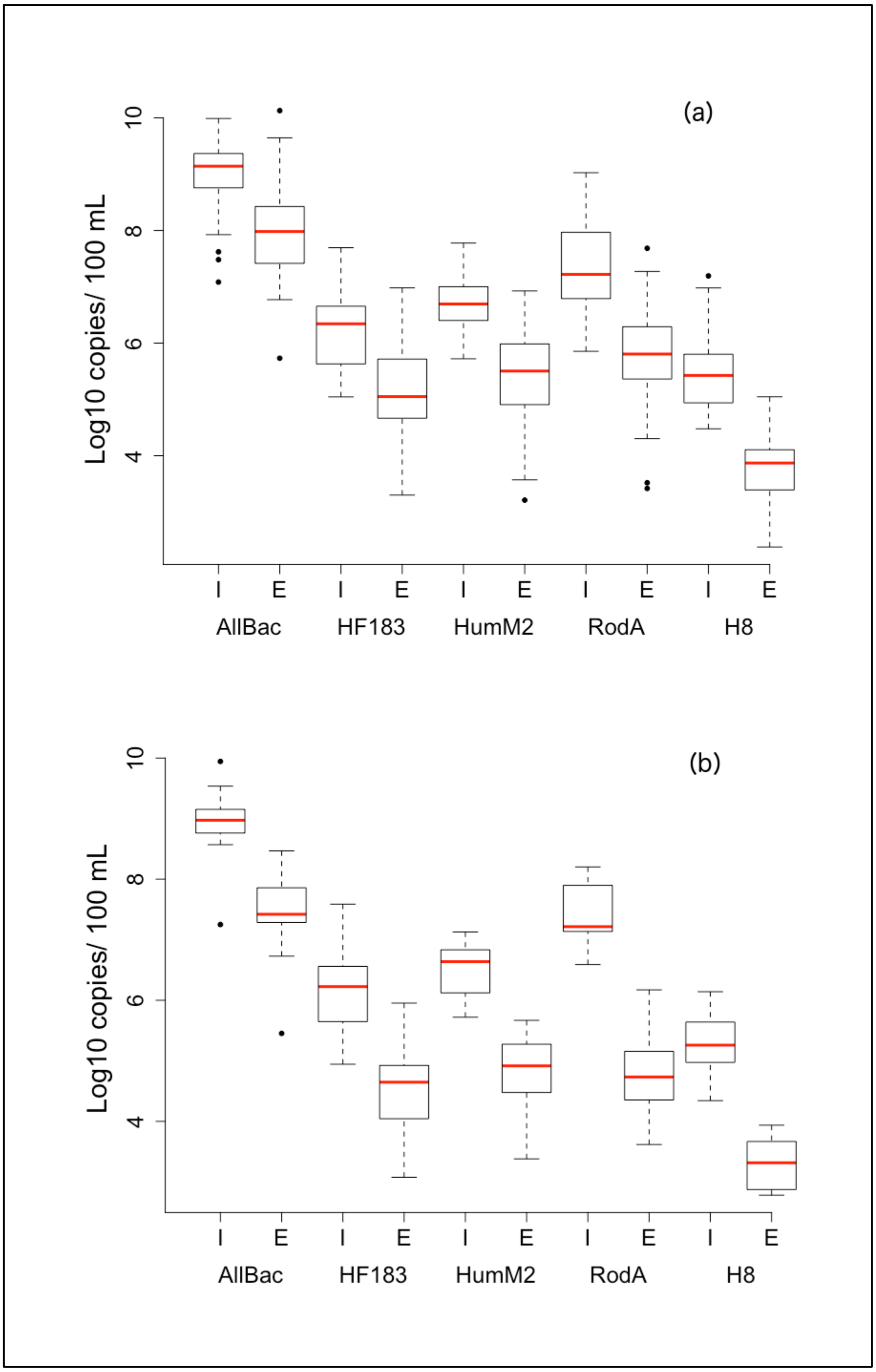


Figure 9 - Abundance of the five genetic faecal markers expressed as gene copies per 100 mL of influent (I) and final effluent (E) samples. Plot (a) is for the small WWTPs (n = 48 influent, n = 48 final effluent), plot (b) is for the larger WWTPs (n = 12 influent, n = 12 final effluent).

However, when influent and effluent sample data were pooled, a significant difference in the abundance of all markers, except AllBac, was observed at the small WWTP compared with the larger WWTPs (Welch's two sample t-test, p = 0.001 – 0.02). Also, a significant difference in all marker abundances was detected in pooled samples collected in the summer versus the winter (Welch's two sample t-test, p = 0.004 – 0.03). This observed seasonal effect is relevant for water quality managers because it draws into question the possible reliability of such markers for exposure assessments in regions with pronounced seasonal differences. To explore the influence of seasonal factors on faecal markers for describing the WWTP performance, summer and winter samples were segregated for all proceeding analysis.

4.3.3 Removal of faecal markers by treatment

The median removal rates of the human-associated markers ranged from $\log_{10}$ 1.3 for HF183 to $\log_{10}$ 1.8 for H8, across all WWTPs. For the non-specific markers, the median removal rates were $\log_{10}$ 1.2 for AllBac and $\log_{10}$ 1.7 for RodA. The difference between the *Bacteroides* and *E. coli* markers might be attributed to variations in temperature and exposure to sunlight, both which change seasonally in the UK.

This speculation was explored by applying clustering algorithms to the final effluent data collected in summer months and comparing it to data collected during the winter months. There was a significant correlation between removal rates of the human-associated *Bacteroides* markers (Pearson's rho = 0.6, p = 1.2e^{-6}) and median removal rates also were similar (i.e., $\log_{10}$ 1.3 versus $\log_{10}$ 1.4). However, less convincing and non-significant relationships were observed between total and human-associated *Bacteroides* markers (Pearson's rho = 0.2, p >0.1). This is consistent with previous observations of changes in the abundance of human-associated and non-specific genetic markers targeting *Bacteroides*, pre and post wastewater treatment (Mayer et al., 2016). In contrast, the correlation between the removal rates of H8 and RodA was particularly strong (Pearson's rho = 0.82, p = 3.7e^{-15}). It should be noted that on four sampling occasions the abundance of faecal markers in final effluents exceeded influent levels, implying a negative removal rate. The same trend also was seen for chemical parameters (see Table 1). Generally, however, it should be noted that an inherent limitation of molecular quantification is indistinction between DNA from living and dead cells, or, indeed free DNA. Thus, observed trends may not be an accurate reflection of bacterial abundances resulting in potential human exposure to faecal pathogens.

4.3.4 *Hierarchical clustering shows seasonal marker trends*

The abundances of all faecal markers in the WWTPS as well as final effluent chemical concentrations are summarised in Figure 10, grouped by experimental category (see section 4.2.1 for definitions). Data collected during the summer and winter months have been separated. Dendographs displaying Ward clustering show that the human-associated markers cluster together in summer samples, whereas in winter samples they do not. Likewise, the non-specific genetic markers cluster together in the summer, but they do not in the winter samples.

Summer patterns might be explained, at least in part, by the fact that non-specific markers tend to cluster closely to performance measures, such as particulate matter, tCOD and TSS. This may be because of excessive microbial growth at SF and RBC plants leading to sloughing of biofilms, containing a proportionally higher number of AllBac and RodA genes. This speculation is supported by the greater copy numbers of non-specific markers in the effluent of 50-125_SF and 125-250_SF WWTPs compared to human-associated markers in summer. However, this speculation could only be confirmed by metagenomic analysis of the appropriate biofilms, which was beyond the scope of this study.

WWTPs within the category 50-125_SF produced noticeably poorer quality effluent in the summer than all other types of WWTP. Interestingly, faecal marker abundances in the effluent appear to be higher than for the 125_250_SF WWTP category for all human-associated markers, although the differences were not significant ($p > 0.05$).

The difference in effluent quality between the small and the larger WWTPs is obvious from visual inspection of the heat maps. For example, the presence of a discharge limit for NH_4-N at the larger works is particularly clear in the data. Such observations alone, however, should not be used to draw conclusions about the dominant factors influencing system performance. For example, none of the WWTPs are actively controlled to remove faecal markers, yet there is a significant difference between the effluent quality and the removal rates measured at small and larger WWTPs. This is irrespective of season and otherwise could be explained by close clustering of certain managed parameters (e.g. tCOD) and the genetic faecal markers. This did not occur and leads us to consider, again, the possible phenomena whereby faecal markers targeting total copy numbers of *Bacteroides* and *E. coli* are closely related to solids removal efficiency, whereas human-associated markers are not.

In winter, as biological activity slows down, faecal marker trends become less clear, with one obvious exception. RodA, targeting total *E. coli* and H8 targeting human-associated *E. coli*, cluster together and appear independent of all other parameters. This may be a result of the effect of temperature on microbial inactivation, of which *E. coli* may be particularly sensitive (Pachepsky et al., 2014). Alternatively, it could result from the effects of sunlight levels on photolytic cell degradation. The degradation rate of *E. coli* in surface waters can be sensitive to sunlight levels, although not always as sensitive as other organisms, such as *Bacteroides* (Noble et al., 2004). When light is limited, the decay of *Bacteroides* is biphasic and generally slow (Green et al., 2011). Assuming that what is observed in surface waters can be extended to WWTPs, this explanation becomes plausible when comparing seasonal gene copy numbers. There is no significant difference between the final effluent counts of human-associated *Bacteroides* in the summer or winter samples ($p > 0.1$), whereas, the difference is significant for H8 (Welch's two-sample t-test, $p = 0.04$).

This observation is potentially important because it implies that in winter conditions, the abundance of *Bacteroides* in effluent samples (which generally are more abundant; see Figure 9) is proportionally greater than in summer. In other words, *Bacteroides* markers accentuate the poor performance of WWTPs in winter. In contrast, *E. coli* markers provide an unrepresentative view of treatment performance because their decay may be less affected by levels of sunlight. This is particularly noticeable at the small WWTPs, whose treatment performance and stability are seasonally as well as generally more inconsistent than larger WWTPs.

It appears that *Bacteroides* markers can provide additional insight into the potential ecological impact of a wastewater discharge, as well as the overall treatment performance of a WWTP. To confirm the usefulness of these observations for understanding small WWTPs, unsupervised clustering analyses were applied to the effluent dataset.

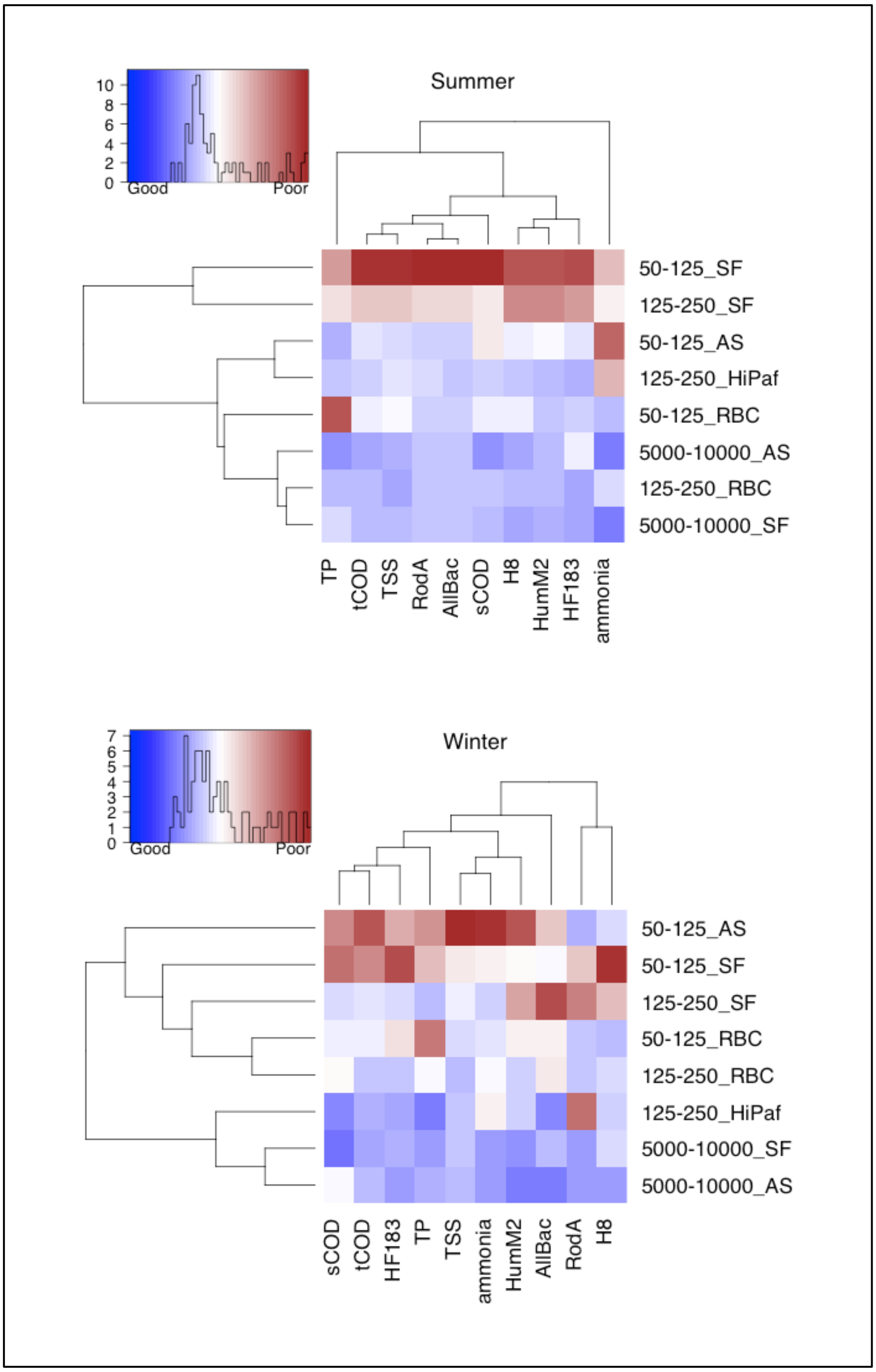


Figure 10 - Heat maps showing the abundance of genetic faecal markers and the concentration of chemical parameters in the final effluent of the WWTPs, grouped by experimental category (see section 4.2.1 for definitions). Data for summer refers to samples collected in June and August, data for winter refers to samples collected in December and February. Dendographs show the output of Ward clustering. Inset graphs show histograms of the scaled datasets where 'Good' is a low concentration and 'Poor' is a high concentration, referring to the quality of the final effluent. The reader should note that the summer and winter datasets were scaled independently, and therefore, heatmap colours should not be compared between summer and winter plots. 'sCOD' is soluble chemical oxygen demand; 'tCOD' is the total chemical oxygen demand; 'TP is total phosphorus; 'TSS' is the total suspended solids.

4.3.5 Vector analysis and partitioning support the use of human-associated markers

The role of the genetic faecal markers as descriptors of variance in final effluent quality between WWTPs is shown in Figure 11. In the summer, the variable loadings attributed to the first principle component (56% of variance) are dominated by the human-associated faecal markers, which all have similar loadings. However, in winter, the dominant loadings against the first principle component (37% of variance) are provided by the *Bacteroides* markers and, interestingly, the loading is approximately equal across the human-associated and the non-specific markers. This further confirms the potential usefulness of *Bacteroides* markers for describing treatment performance trends across groups of WWTPs.

To explore the use of the markers for describing the behaviour of individual WWTPs, or groups of WWTPs within a larger network, k-medians clustering was applied (see colours in Figure 11). According to variance, effluent quality as described by the faecal markers cannot be grouped in equal clusters. This suggests that a large proportion of the WWTPs are indistinguishable in terms of effluent quality variance. However, some trends are clear. For example, samples collected at the smallest WWTPs (50-125 PE) cluster together, across both season (red coloured points in summer and green coloured points in winter; Figure 11). The implication is that the effluent quality of such plants is similar across all faecal markers and distinct from the slightly larger WWTPs (125-250). This corroborates the findings of Chapter 3 that the influence of size on the performance and stability of small WWTPs may be more important than previously recognised.

The function of k-medians clustering is that clusters are identified by the least variance between data points. The variance of each of the faecal markers across each of the clusters for summer and winter was calculated to test which marker best describes the clustering. In other words, which marker best describes the differences in final effluent quality observed across the different WWTPs by having the lowest within-cluster variance. In summer, the lowest variance in two of the three clusters was seen with the human-associated faecal markers. However, one of the clusters can be best described by the variance of RodA abundance. In winter, variance within each cluster is best described by variance of the markers targeting *Bacteroides*.

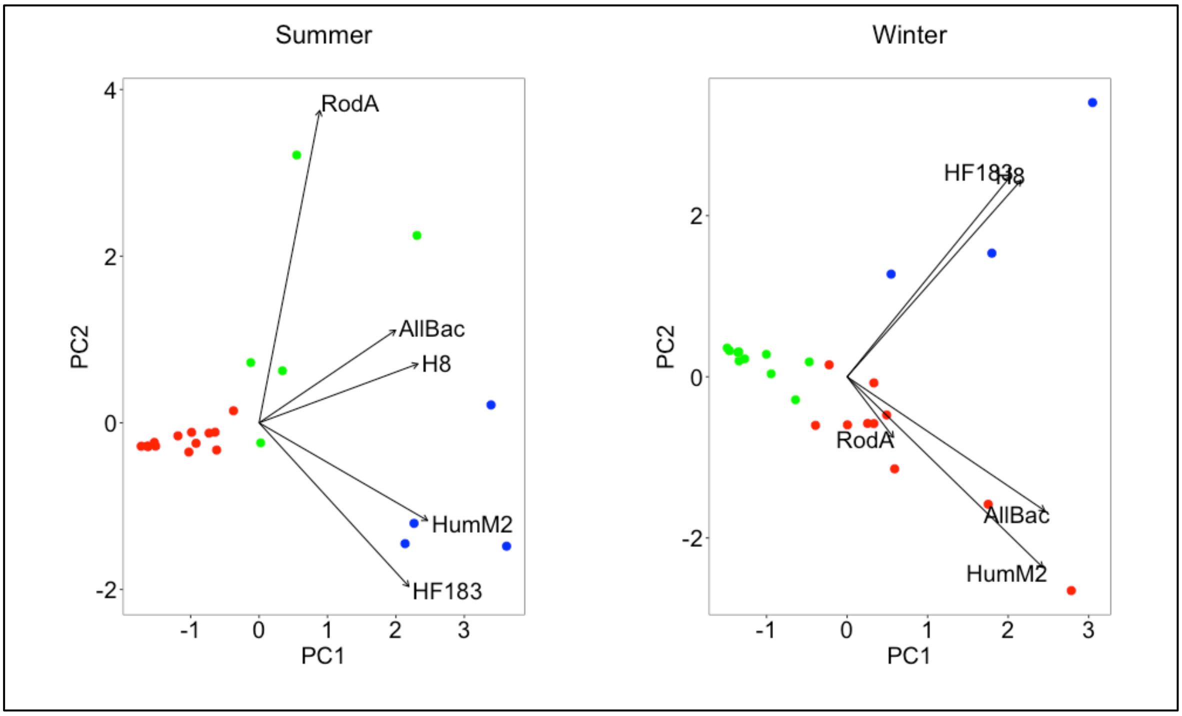


Figure 11 - Analysis of principle components combined with k-medians clustering of final effluent data collected in summer months and winter months. Vectors indicate the direction of the parameter effect, as derived by principle component analysis. Colours show the k-median clusters.

4.4 Conclusion

This study sought to investigate the use of genetic faecal markers to improve understanding of differences between the treatment performance of small WWTPs. During summer operation, human-associated markers appear to be best for describing WWTP treatment performance and can potentially provide useful insights beyond chemical metrics. In winter, *Bacteroides* markers appear to be better than *E. coli* markers, possibly because of the susceptibility of *E. coli* to changes in temperature and sunlight, although this speculation must be validated in directed experiments. There was a significant difference in marker abundances measured at the small WWTPs compared to the larger WWTPs, and there was a significant difference between abundances measured in the summer and winter.

It is clear that genetic faecal markers can provide wastewater managers useful insights on the treatment performance and variance of small WWTPs. The *Bacteroides* markers provided the most representative description of differences between different WWTPs and so are recommended, especially the human-specific markers. However, seasonal effects on marker fate suggest faecal markers should be used with caution because the best marker appears to differ between seasons, even for the same types of WWTP, which will impact their utility in places with pronounced seasonal variations, such as in temperature and sunlight.

Chapter 5. An Inverse Solution to the Problem of Predicting Dry Weather Flows at Small Wastewater Treatment Plants

5.1 Introduction

The effective management of wastewater discharges from small WWTPs may be critical for preserving surface water quality. As established in Chapters 3 and 4, understanding the performance and stability of small systems is of particular concern as the ecological impact of such systems may be underestimated. It is important, therefore, to identify how such systems function and what influences their performance reliability, especially compared to larger, centralised WWTPs. It has been shown that the reliability of small treatment systems can be affected by multiple factors, some of which may be unique to smaller contexts, including the susceptibility of smaller WWTPs to receive variable flows (Capodaglio et al., 2017). Sudden fluctuations in pollutant load – typically referred to as, shock loading - can impact the performance of treatment systems and effluent quality, which may in turn, impact the receiving waters. Within this context, the need for high resolution flow data is clear, particularly for small WWTPs. However, small treatment plants are not routinely monitored (EA, 2018b) and, as a result, there is a lack of data, including flow rates.

When such information is missing for larger systems, modelling can be used to infer data or run future scenarios. This is because the prediction of wastewater influent characteristics is well established and can be used for generating inputs for process simulators, such as the Benchmark Simulation Model (BSM) platform. Overcoming the high costs associated with experimental data collection has resulted in the extensive use of such models for WWTP optimisation (Jeppsson et al., 2013). Influent generators may employ deterministic approaches or, as is most common, use Fourier-based dynamics to estimate diurnal profiles from daily average flows or to infill missing data (Langergraber et al., 2008; Mannina et al., 2011). Phenomenological models have also been developed (Gernaey et al., 2005, 2006, 2011), primarily for use in conjunction with the BSM series. These models have evolved over the past twenty years from a simple set of flow modules to a complex system of models with multiple, inter-connecting facets. In terms of data-driven approaches, several attempts, with mixed success, have been made to apply machine learning algorithms to infer influent characteristic timeseries from historical data (Pai et al., 2011; Cheng et al., 2018). Common techniques such as neural networks or gradient boosting machines are typically employed, for both of which, the accuracy of prediction is linked to the size of the input dataset. Finally, commercially available WWTP process models (e.g., SIMBA, Visual Hydraulics, STOAT) often include influent characteristic generators which begin with flow profile predictions; the methodology is not always clear, resulting in a 'black-box' scenario for modellers.

Whilst well established in some cases, the array of existing approaches for predicting or inferring influent flow data may not be suitable for use in small-scale systems. There are two main reasons:

1) The focus of existing models is on data generation for optimising the process management of large WWTPs. For example, the phenomenological model of Gernaey et al. (2011) inputs to BSM2 which typically defines influent characteristics for a population equivalent (PE) of 100,000. It may not be appropriate to assume that the influent characteristics or the WWTP response is the same at small systems as it is at larger systems.
2) Existing models generally rely on large and complex datasets. Whether the model is driven by a deterministic representation of a complex physical processes (i.e., the WWTP catchment), or machine learning algorithms, the necessary data are unlikely to be available for small WWTPs operating in rural and remote locations.

Thus, there is a need for a new approach to predicting flows at small WWTPs, which is the aim of this chapter. The inherent variability of flows received by small WWTPs, particularly under dry weather conditions, and the likely, rapid return to a consistent diurnal pattern following a period of wet weather, means that predicting their flow rates should be considered independently from larger WWTPs. Whereas, the flow rate behaviour of small WWTPs under wet weather conditions is complex and difficult to predict due to the 'flashiness' of the catchment and short sewer network lengths. Thus, the scope of this chapter is the prediction of dry weather flows received by small WWTPs.

5.2 Modelling Approach

The approach to modelling employed is one of induction, whereby the model is used for the extrapolation of measured data in time and space (Beven, 2012). In contrast to other widely held views of modelling, data-based mechanistic modelling (Young &

Beven, 1994) is not concerned with achieving the best possible reflection of the physical processes involved in determining wastewater fluxes. It is wholly empirical, relating a series of data inputs directly to a series of outputs. Such an approach has been used extensively in hydrological modelling (e.g., Farmer et al., 2003; Kirchner, 2009), but has not previously been applied to the issue of assessing wastewater flow characteristics. The simplicity of the approach is attractive, given the large number of unmonitored WWTPs across the UK. Thus, the work of Kirchner (2009) is of particular interest, showing that a single equation rainfall-runoff model can predict flows as accurately as other more complex and highly parametrised models, especially when solved by analytical inversion. This was the chosen approach here; i.e., a simple, single-function solution to determine the flowrates typically received by small WWTPs.

A linear reservoir function was used to represent the total wastewater flow rate, as defined by Equation 4. Q is the timeseries of predicted discharges from the sewer system into the WWTP, Q_0 is the flow rate at time t = 0, R_t is the predicted human generated input flux at time t (see Equations 5 to 7 for derivation of R), dt is the timestep interval and τ is the travel time through the sewer network, commonly referred to in hydrological modelling as the residence or the storage time (see Equations 8 and 9 for application of τ).

$$Q = Q_0 * e^{-(dt/\tau)} + R_t * [1 - e^{-(dt/\tau)}] \quad (4)$$

The flow rates observed under wet and dry weather conditions were treated separately because it was expected that flow rates would behave differently under each condition. More specifically, it was expected that under dry conditions, a ‘predictable’ pattern could be identified, based on consistent human behaviour.

5.2.1 Definition of dry weather flow rates

Flow rates under dry weather conditions were determined for twenty-one small WWTPs in the NE of England (Table 5). Fifteen-minute interval flow rates for the period 01 August 2013 and 31 July 2016 were obtained from Monitoring Compliant Certification flow meters (EA, 2014). Dry weather flows were defined as the flow rate occurring when there had been no measured rainfall for the previous forty-eight hours; i.e., where the rain radar recording was zero. This approach was chosen for simplicity and to facilitate linking the rainfall data and the measured flow data. It was deemed appropriate for small catchments due to the relatively short travel times through the sewer network. Diurnal flow profiles for each WWTP were created by calculating the mean flow rate at each fifteen-minute timestep.

Site	Population Equivalent	Sewer Network Length (m)	Longest convex hull axis (m)
Barrasford WWTP	237	2331	365
Blanchland WWTP	100	774	346
Butterhaugh WWTP	177	3861	981
Carlton-in-Cleveland WWTP	244	3362	1055
Fir Tree WWTP	265	2248	485
Garrigill WWTP	132	2402	1192
Glanton WWTP	208	4258	638
Holy Island WWTP	225	5331	720
Ingleby Greenhow WWTP	200	2497	1080
Low Worsall WWTP	20	2595	535
Matfen WWTP	213	4389	1851
Powburn WWTP	184	1915	770
Romaldkirk WWTP	154	2226	662
Rookhope WWTP	211	3017	1425
Scots Gap WWTP	221	4363	1839
Snitter WWTP	36	2261	1428
Wall WWTP	261	2932	677
West Woodburn WWTP	176	3913	730
Whittingham WWTP	231	2568	686
Whorlton WWTP	183	1948	603
Winston WWTP	254	3788	947

Table 5 - List of WWTPs used for modelling with the relevant population equivalents, sewer network lengths and the length of the longest axis of a convex hull drawn around the sewer endpoints. This measure has been included to provide an indication of the maximum distance of travel within the catchment, to the WWTP. Further explanation is provided in section 5.2.5.

5.2.2 Measured dry weather flows

Once dry weather flows were isolated from the total flow rates, mean diurnal profiles were calculated for each WWTP. The measured dry weather flow rate data was cleaned to remove the effects of potential flow meter failure. Datasets for each WWTP were checked to ensure no more than 30% of the meter readings were missing, which would imply the on-site flow meter was unreliable. Unrealistically high flow rates also were removed, and replacement values were inferred by linear interpolation using the zoo package in R (Zeileis & Grothendieck, 2015), with the replacement value chosen being the closest to the data extreme. Unrealistic flow rates for a particular WWTP were defined as occurring when the difference between two adjacent flow points was greater than the mean difference between adjacent flows points, recorded at that WWTP. This approach was chosen because such ‘flashiness’ is typically associated with individual storm events and would not be relevant under dry weather conditions, or detectable when flows at each timestep were averaged. Therefore, any extreme outliers could be considered atypical and likely be a result of flow meter failure. The effects of infiltration on the dry weather flow were considered by subtracting the mean minimum flow observed over a diurnal period from the flow rate observed at each timestep. Thus, the base dry weather flow rate for each model WWTP was 0 m/s. The processed, dry-weather diurnal flow profiles are shown in Figure 12.

The median dry weather flow rate across the twenty-one WWTPs was 0.29 L/s which implies a median daily per capita wastewater contribution of 134 L. The standard deviation ranged from 0.01 L/s, which was for Garrigill WWTP, to 0.44 L/s, which was for Matfen WWTP. This is within the range average per capita water consumption values for the UK (CC Water, 2019), which can be used as a metric for dry weather flow contribution. The highest median flow rate was observed at Matfen WWTP; however, the highest expected flow rate might be for the WWTP with the largest PE, which was Fir Tree WWTP. Under such circumstances, it is likely that a non-domestic flow contribution is dominant within the WWTP catchment. This is the case for Matfen WWTP and, specifically, flows were probably dominated by a large local hotel. For most WWTPs, such a feature may not be relevant, however, in small WWTP catchments, the relative contribution of a single facility, such as a hotel, can have a dramatic effect on the wastewater flux. Whilst each WWTP clearly has a unique flow signature, it also is clear that the majority of WWTPs follow a similar diurnal profile under dry weather conditions, typically consisting of a steep rising limb to a morning flow peak; a gentle falling limb to daytime base flows and a less steep rising limb to a second flow peak in the afternoon, falling to overall base flows shortly after mid-night.

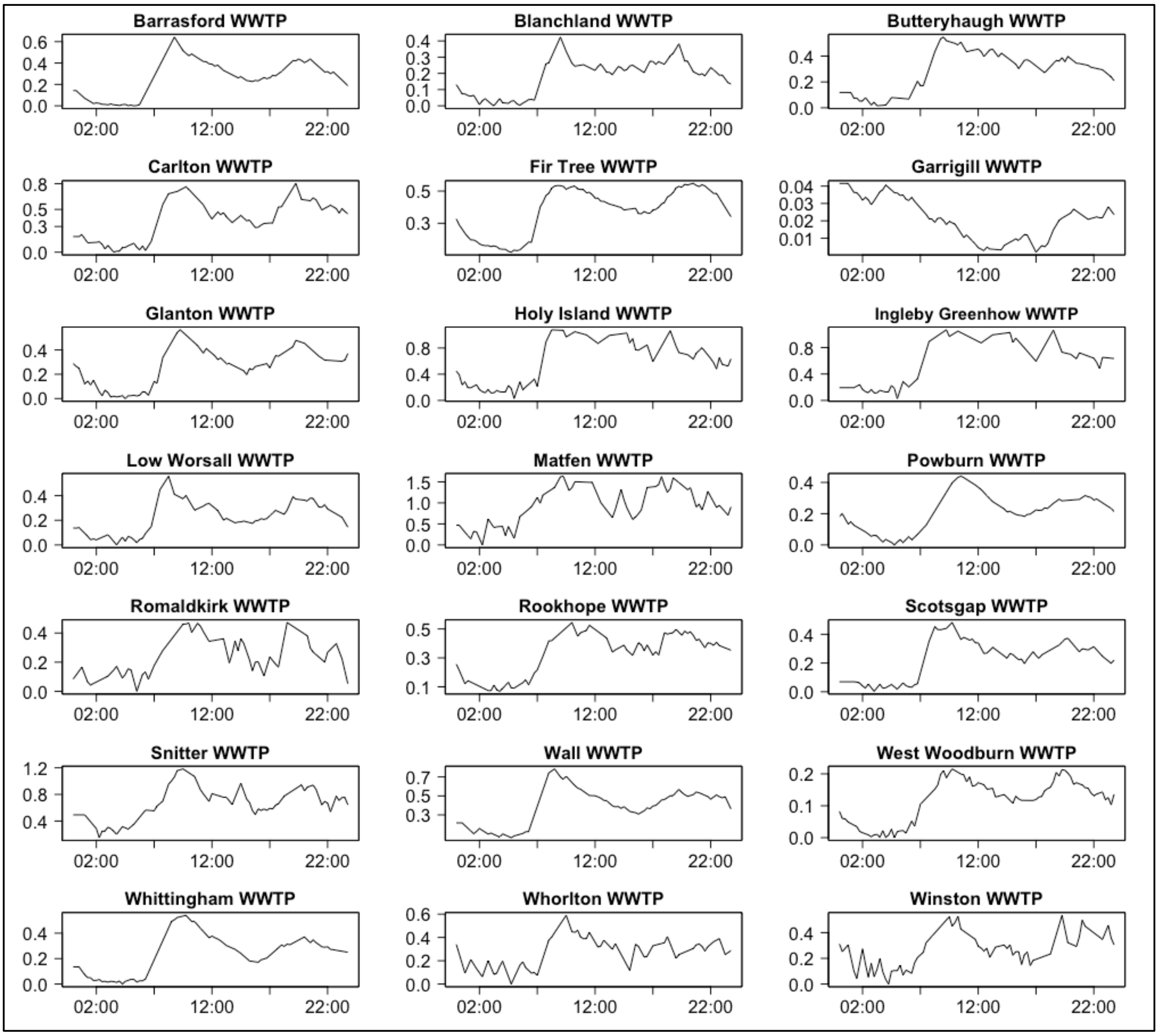


Figure 12 - The mean diurnal flow rate profiles under dry weather conditions, after data cleaning.

5.2.3 Rainfall data processing

Rainfall data was acquired from Northumbrian Water Ltd. for the period 01 August 2013 to 31 July 2016. The data included rainfall depths recorded at five-minutely intervals at a km^2 spatial resolution. For each WWTP network, the grid-squares that covered the spatial extent of the sewerage system were determined using maptools and Raster packages in R (Bivand & Lewin-Koh, 2019; Hijmans, 2019). Rainfall data for a particular grid-square was included if more than 30% of the total sewer network length passed through the grid-square and if that sewer was not receiving only foul flows (i.e., human-derived. Separated sewer systems carry either sewage from buildings, or surface water from rainfall run-off. Those carrying sewage from building, only, are termed, ‘foul sewers’). To align the rainfall and measured flow data, the rainfall data was converted to fifteen-minutely interval by summing the three prior readings. For example, the depth at 03:15 was the sum of the depths recorded at 03:05, 03:10 and 03:15. Thus, it was assumed that the rainfall was even distributed across the fifteen-minute time period. There were no missing values in the raw or processed data. To convert the rainfall depths into volumes that fell specifically within each WWTP catchment, the depths were multiplied by the area covered by the sewerage infrastructure serving each WWTP. The area was calculated by drawing a ten-meter buffer around the sewers using the maptools package in R.

5.2.4 Additional catchment characteristics

The length of the wastewater collection network within each WWTP catchment was required to estimate the travel time of wastewater through the sewer. It was calculated from ESRI shapefiles provided by Northumbrian Water using R. The sewer length included combined, foul and surface water sewer types. Overflow and emergency overflow pipes and culverted watercourses were removed from the database as they do not typically contribute to the flows received by a WWTP. The length of the sewerage network in the catchments used for modelling ranged from 774 m to 5331 m with a median network length of 2595 m (Table 5). The length of the longest axis of a convex hull drawn around the end points of the sewers in each catchment was calculated using QGIS 3.0 (QGIS Development Team, 2019). This was used in conjunction with the sewer length to determine the travel time of wastewater through the network (see section 5.3.2). The length of the longest axis of the convex hull was used to provide an indication of the maximum distance within the sewer catchment to the WWTP. This is a particularly important consideration for small WWTPs because the shape of the network may be linear (i.e., several sewer pipes flowing near-parallel to one another with few lateral pipes), or it may be more radial (i.e., sewer pipes converge from multiple directions, on or close to, a single point which leads to the WWTP).

The PE for each WWTP was provided by Northumbrian Water Limited and combined with the mean, daily, per capita water consumption in the UK (CC Water, 2019) to give the total human-generated wastewater flux over a diurnal period.

5.3 Predicting Flows Under Dry Weather Conditions

5.3.1 Model concept

Whilst a linear reservoir function is designed to be a simplistic representation of a catchment, complexity in solving Equation 4 can arise from parameter interactions that make it more difficult to define input sources. Thus, an inverse approach was taken. Philosophically, the approach is analogous to the Generalised Likelihood Uncertainty Estimation (GLUE) commonly associated with addressing problems of equifinality in hydrological modelling (Beven & Binley, 1992, 2014). Here, it was anticipated that a similar problem would be encountered whereby multiple combinations of the input parameters could result in similar outputs. To overcome this, a prior distribution was defined for each input parameter. Monte Carlo simulations were used to sample the distributions for parameter values, which generated multiple model simulations. The best performing simulations for each WWTP were combined to create a representative dry-weather flow prediction model that was relevant to and representative of multiple WWTPs.

5.3.2 Definition of source terms

The dry weather diurnal flow profiles shown in Figure 12 show the flow rates observed at each of the model WWTPs and, therefore, the average pattern of dry weather flow contributions. Considering also travel time of flows through the sewer network, this forms the premise for the dry weather component of the model. The majority of the WWTPs share a similar diurnal flow profile, including a sharp rising limb between 06:00 and 08:00 followed by a less steep recession limb where upon flow recedes to day-time base flows. A less pronounced peak can be observed in the afternoon with the recession limb eventually receding completely to base dry weather flows at approximately 04:00. The pattern is a result of the broadly consistent shape of human-derived flow contributions throughout a typical day. Thus, the input flux driving the dry-weather flow profile can be simplified to consist of three parcels of flow (Figure 13).

An inverse approach to solving the linear reservoir function requires the definition of upper and lower bounds for each input parameter to generate a prior distribution. The magnitude of the wastewater input flux was defined by the following set of equations which correspond to Figure 13. Throughout, R is the predicted timeseries of human generated input flux to the sewer system, measured in L/s; and α is the total human generated input over the duration of the simulation (in this case, 24 hours at fifteen-minute timesteps) and is measured in L. The population equivalents for each WWTP were multiplied by the average per capita water consumption for the UK, which was 149 L (CC Water, 2019). This defined α.

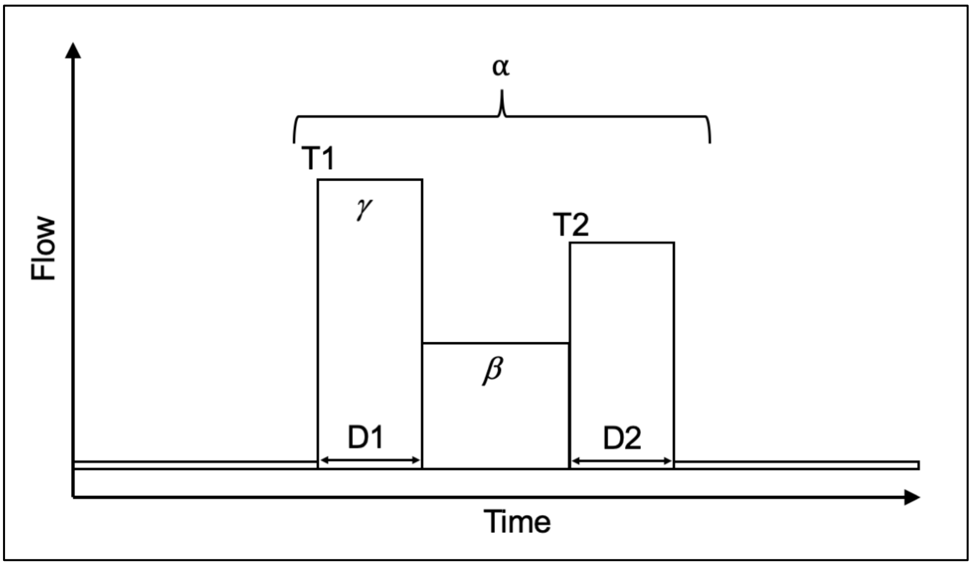


Figure 13 - A simplistic, schematic representation of the human-derived wastewater flow contributions under dry-weather conditions. Where, α is the total flow contribution; T1 is the start time for the first flow peak and T2 the start of the second; D1 and D2 are the duration of the first and second flow peaks, respectively; β is the proportion of α that is assigned to the day-time base flows and γ is the proportion of the peak flows assigned to the first flow peak.

At time t < T1, R is assumed to be equal to zero because α is likely to be equal to zero (the long-term effects of infiltration are considered, as previously described); T1 is the start of the first flow peak and t begins at 00:00. The upper and lower limits for T1 simulations were chosen by observation of the measured dry-weather diurnal profiles, as shown in Figure 12.

At time, T1 < t < (T1 + D1), where D1 is the duration of the first flow peak; γ is the proportion of the sum of the two peak flows that is assigned to the first peak (i.e., morning peak); β is the fraction of α that is assigned to period of time between the two flow peaks, i.e., the flow between (T1+ D1) and t2 and is considered to be the day-time base flows:

$$R = \frac{(\alpha - (\alpha * \beta)) * \gamma}{D1} \qquad (5)$$

At time, T2 > t > (T1 + D1), where T2 is the start of the second flow peak, and all other parameters are as previously defined:

$$R = \frac{\alpha * \beta}{T2-(T1+D1)} \qquad (6)$$

At time, T2 < t < (T2 + D2), where D2 is the duration of the second flow peak and all other parameters are as previously defined:

$$R = \frac{\left(\alpha-(\alpha * \beta)\right) * (1-\gamma)}{D2} \qquad (7)$$

Finally, where t > (T2 + D2), R was assumed to tend towards zero as an exponential decay.

The timeseries of human-generated input flux, R, was used as an input to the linear reservoir function (Equation 4), which includes the parameter τ, which is the travel time through the sewer network. A form of Manning's equation was used to inform the prior distribution:

$$\tau = \frac{l}{\left(\frac{1}{n} * r^{\frac{2}{3}} * S^{\frac{1}{2}}\right)} \qquad (8)$$

For Equation 8, n is Manning's roughness coefficient which was set to 0.012, assuming concrete sewers; r is the hydraulic radius calculated from the wetted perimeter and assuming that the pipe diameter was 150 mm, and S is the gradient of the slope and assumed to the 1:150, which is the minimum guideline gradient for 150 mm sewers (BSI, 2017); l is a factor which considers the ratio of the length of the sewer network within the WWTP catchment against the length of the longest axis of a convex hull drawn around the sewer endpoints (Equation 9), where h is the length of the convex hull axis and L is sewer network length. Thus, it was assumed that the velocity of the wastewater flows was consistent across all networks and constant through each network. The travel time, then, is dependant only on parameter, *l*. This is important because alternative methods for calculating flow velocities are dependent on knowing the flow rate, which would not be available for unmonitored WWTPs, for which this model is designed.

$$l = \frac{L}{\sqrt{L/h}} \qquad (9)$$

5.3.3 Description of prior distributions

In order to pursue the aim of developing a simple, representative flow model, consistent distributions were used across all WWTP models for the majority of input parameters; only α and τ were the only defined WWTP-specific terms. Thus, variable source terms were the population equivalents, the sewer network lengths and the length of the longest axis of a convex hull drawn around the sewer endpoints, for each catchment (Table 5). The upper and lower limits of the prior distributions for each parameter are shown in Table 6. It should be noted that the ranges shown are not the ranges that generated the best performing simulations; the optimised parameter set is summarised in section 5.3.6

The broad range for γ was chosen to accommodate scenarios where the falling limb from the first flow peak extends over such a long time period that there is little distinction between the first and second flow peaks. The lower limit for β also was set to accommodate this scenario.

Parameter	Description	Units	Lower limit definition	Upper limit definition
α	Total human input	L	0.5 * (PE * 149)	1.5 * (PE * 149)
β	Proportion of α assigned to base flows	%	10	50
γ	Proportion of peak flows assign to first peak	%	20	70
T1	Start time of first flow peak	Time	05:00	09:00
T2	Start time of second flow peak	Time	14:00	19:00
D1	Duration of first flow peak	Hours	0.5	4
D2	Duration of second flow peak	Hours	2	6
τ	Travel time through sewer network	Hours	0.5 * τ	1.5 * τ

Table 6 - Upper and lower limits for prior distributions used for Monte Carlo simulations for the development of the dry-weather flow model component.

5.3.4 Assessing input parameter sensitivity

Nash-Sutcliffe Efficiency (NSE) was used to compare the performance of each simulated model run against the measured flow rate data for each WWTP. To test the sensitivity of each input parameter to the sampling from the prior distribution, the effect on the overall NSE was considered. This was done for each of the WWTPs used for modelling. By plotting the resultant NSE for each parameter against the sampled values for all model simulations, it was possible to assess the behaviour of each parameter against the model performance in each simulation. Put simply, the more rounded the shape of the plot, the more tightly constrained the effect of the parameter on the model performance.

The sensitivity plots for one million simulations for Fir Tree WWTP are shown in Figure 14 as examples. It is clear from this example, that the total wastewater flux (α) was important, which is expected as several of the other parameters are derived from it (see section 5.3.2). The start times for each of the flow peaks (T1, T2) also were important with clear points at which NSE was maximised, at 06:30 and 16:00, respectively. The skewed nature of the plots for α and D2 suggest that the parameter ranges are not optimal, however, it is sufficiently clear to identify values that might result in the best performing model simulations.

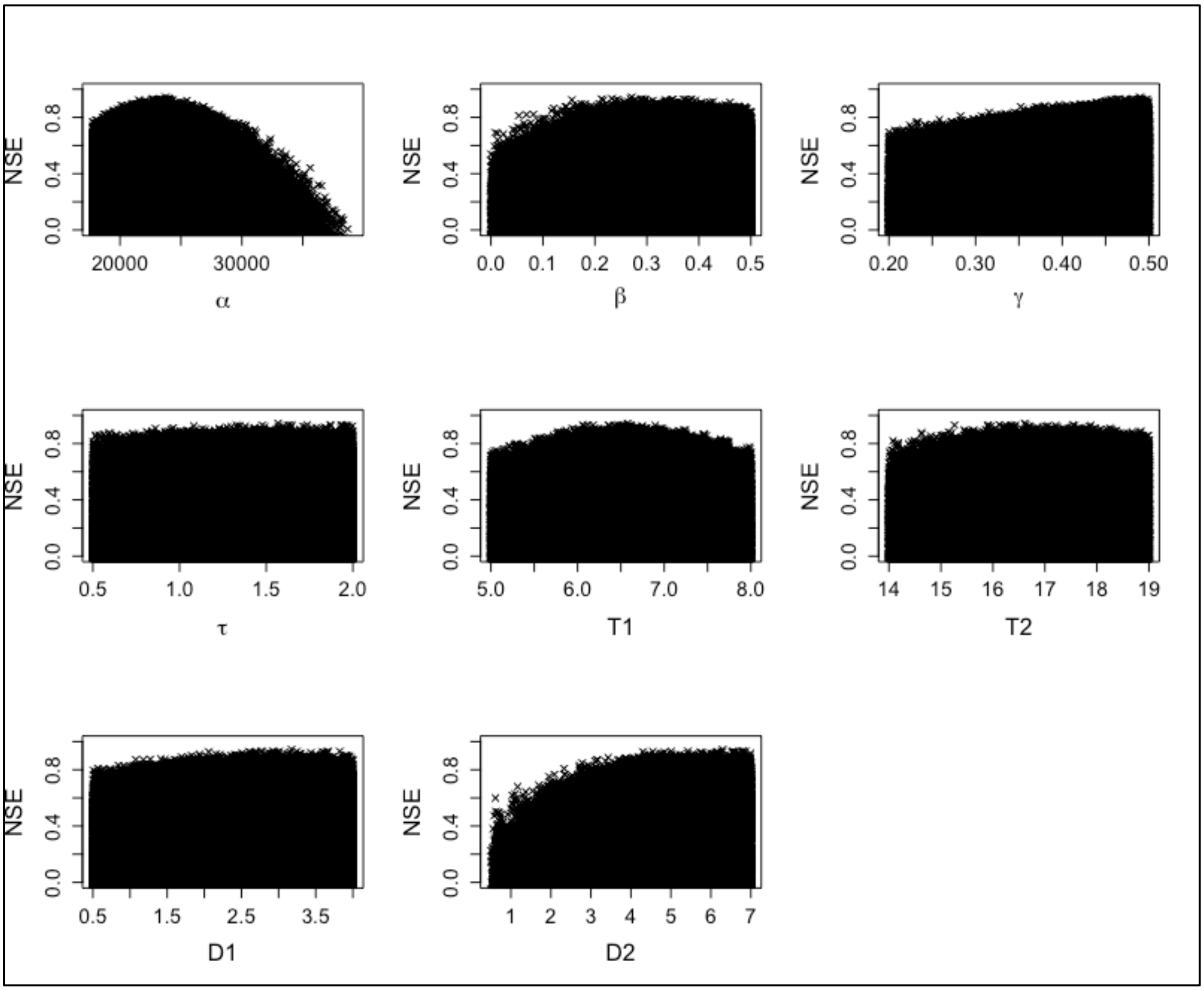


Figure 14 - Example NSE parameter plots for Fir Tree WWTP. NSE is the Nash-Sutcliffe Efficiency and all parameters are as described in Section 5.3.2.

5.3.5 Predicted dry weather flow profiles

Dry weather flow profiles were predicted by calculating the human-derived flow contribution and the flow travel time as inputs to a linear reservoir function. Values for the input parameters were sampled from a prior distribution to generate multiple simulations. An initial model run of ten thousand simulations was carried out to identify any WWTPs for which the model failed to represent the measured flow rates. This was defined as the maximum NSE being below 0.7 and resulted in the following WWTPs being excluded from the modelling process: Garrigill WWTP, Low Worsall WWTP, Matfen WWTP, Snitter WWTP and West Woodburn WWTP. Considering the diurnal flow profiles of these treatment plants under dry weather conditions (Figure 12), it is not surprising the model was unable to meet stringent performance criteria in these cases. Thus, flow rates measured at the other sixteen WWTPs were used to build a representative model.

One million simulations were generated for each of these sixteen WWTPs. The resulting maximum NSE values ranged from 0.77 to 0.94 with a mean maximum NSE of 0.88, suggesting a high-level of prediction accuracy was achievable. As a demonstration of the site-specific model output, the predicted diurnal flow profile for the best performing simulation (max NSE) for Fir Tree WWTP is shown in Figure 15. For diurnal profiles for all WWTPs, see Appendix C. For the majority of the profile, the measured flow falls between the upper (Q75) and lower (Q25) quartiles of the predicted flow (Predicted Q). This is encouraging given the simplicity of the model and the limited range of input parameters. Points where the model appears to be less reliable in this example include the early hours of the morning, where the model over-predicts the flow. This probably results from assumptions made when determining the prior distribution for the travel time (τ).

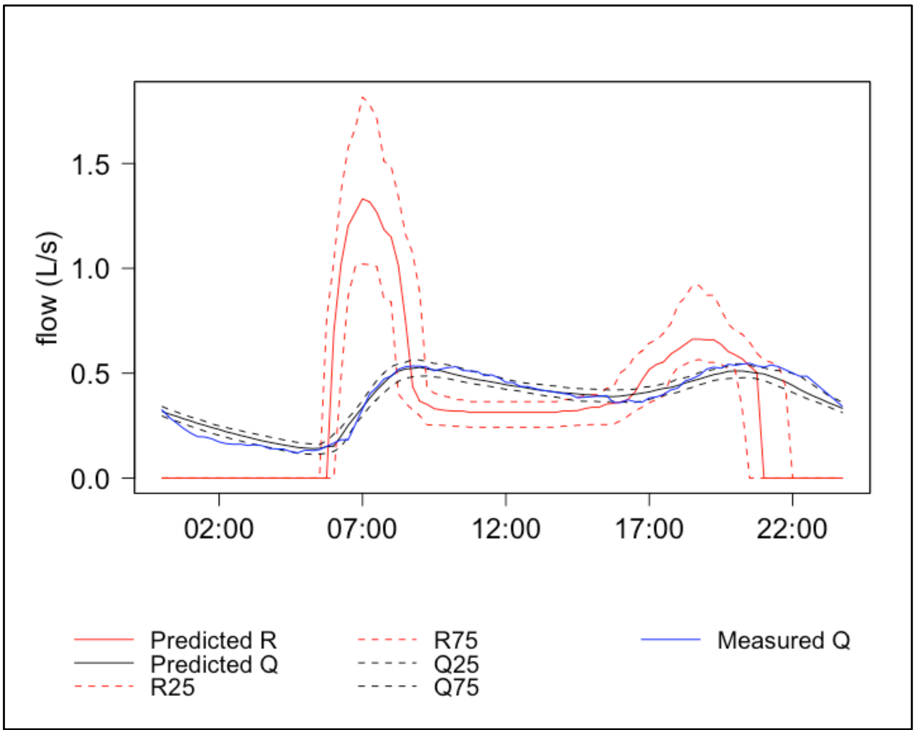


Figure 15 - Predicted diurnal flow profile (Predicted Q) for Fir Tree WWTP plotted with the measured flow rate (Measured Q) and the predicted human-derived flow contribution (Predicted R). Upper and lower quartiles of the predicted profiles are also shown (R25, R75, Q25 and Q75).

5.3.6 Identifying a representative parameter set

One million simulations were run to generate a suite of parameter sets from which representative models were chosen. The model simulations that best represented the measured flow rates were defined as those with the highest NSE score. The shape of the NSE parameter plots (e.g., Figure 14) suggested that the best performing simulations were not likely to be restricted to a small range of parameter values and so a relatively simple approach to optimisation could be employed. Thus, the one hundred model simulations that resulted in the highest NSE score for each WWTP were chosen and consolidated to give sixteen hundred representative model simulations. The maximum NSE for the sixteen hundred model runs ranged between 0.77 (Ingleby Greenhow WWTP) and 0.94 (Barrasford WWTP). To generate a set of parameters that is generally representative of a dry weather flow pattern at small WWTPs, the model simulation which resulted in the median NSE from amongst the sixteen hundred runs was identified. This provided values for the parameters (Table 7) that would be required to calculate the human-generated flow contribution and dry-weather diurnal flow profile for unmonitored small WWTPs. Due to the highly site-specific nature of the network characteristics and the PE, optimal values for the total human input (α) and the travel time (τ) were not included in the optimal parameter set and instead, site-specific prior distributions were used. The parameters are included in Table 7 for completeness. The site-specific prior distributions for α and τ are shown in Table 8.

Parameter	Units	Value at Median NSE
α	L	34,766*
β	%	43
γ	%	51
T1	Time	06:03
T2	Time	18:17
D1	Hours	3.3
D2	Hours	5.6
τ	Hours	2.33*

*Table 7 – Parameter values for the median NSE amongst the top 100 model runs, measured by NSE, across all sixteen WWTPs. These parameter values were used to generate the optimal model parameter set. * Note – optimal values for α and τ were not included in the optimal parameter set and instead, site-specific prior distributions were generated. They are included here, for completeness. See Table 8 for a list of site-specific prior distributions for α and τ.*

The parameter set resulting from the simulation with the highest NSE was not chosen because of the possibility of the high performance being a result of the specific characteristics of the modelled WWTP. By choosing the parameter set associated with the simulation that resulted in the median NSE, it is likely that a more accurate model prediction could be made for WWTPs with a wider range of catchment characteristics.

Site	Prior distribution for α (L)	Prior distribution for τ (hours)
Barrasford WWTP	19744 - 29114	0.23 - 0.69
Blanchland WWTP	13815 - 18189	0.90 - 2.69
Butterhaugh WWTP	21994 - 28244	1.12 - 3.36
Carlton-in-Cleveland WWTP	28930 - 44009	1.01 - 3.03
Fir Tree WWTP	28424 - 36716	0.47 - 1.41
Glanton WWTP	20850 - 27947	0.42 - 1.25
Holy Island WWTP	44250 - 50581	0.23 - 0.68
Ingleby Greenhow WWTP	38449 - 44997	1.08 - 3.23
Powburn WWTP	15657 - 23591	1.73 - 5.18
Romaldkirk WWTP	17246 - 26650	1.03 - 3.08
Rookhope WWTP	25334 - 33516	1.89 - 5.67
Scots Gap WWTP	16908 - 24053	2.17 - 6.51
Wall WWTP	30289 - 39853	0.45 - 1.35
Whittingham WWTP	17983 - 25771	0.52 - 1.55
Whorlton WWTP	19640 - 29292	0.53 - 1.58
Winston WWTP	21462 - 30666	0.67 - 2.00

Table 8 – Site-specific prior distributions for α and τ, for all WWTPs.

It is evident that for some of the WWTPs, the model failed to predict the highly variable nature of some diurnal flow profiles. Whilst these profiles broadly follow the pattern upon which the model was conceptualised, there are fluctuations within this framework. Such variability could be influenced by the positioning of properties along the sewer network or non-domestic sources within the catchment, both which are difficult to quantify.

Thus far, the assessment of model performance has been limited to overall accuracy or observation of profile trends. To further test the performance, a form of cross-validation was carried out on the representative model.

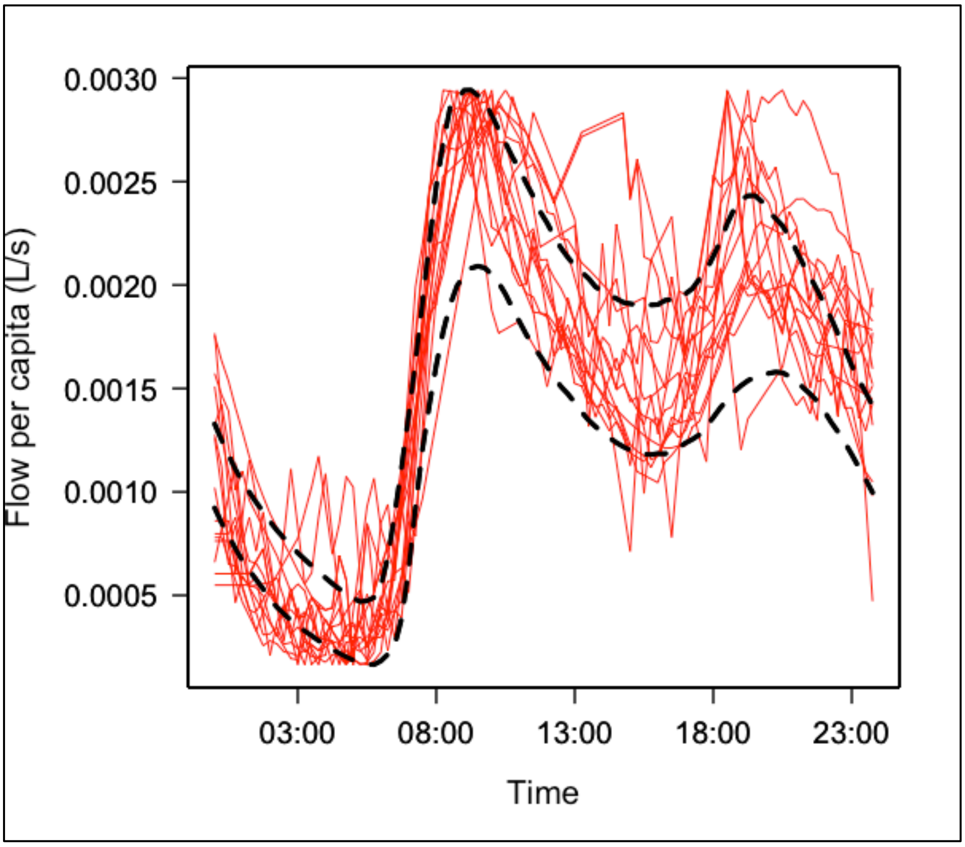


Figure 16 - Measured diurnal dry-weather flow profiles for the sixteen small WWTPs shown as red lines. The black dashed lines are the upper and lower quartiles of the predicted flow profile using the representative parameter set. Flow shown as per capita to accommodate the varying sizes of WWTP.

5.3.7 Cross validation of dry weather model

Conventional methods of cross-validation, such as k-fold cross validation, are not always appropriate for assessing the performance of a timeseries model. This is especially true in this case, where the model outputs are a predicted diurnal profile; i.e., withholding data in sequence would result in a loss of a particular time period. So, to assess how well the representative dry-weather model performs, a novel form of cross-validation was employed. For each WWTP, a new representative parameter set was generated as described in section 5.3.5, but in turn withholding each WWTP. For example, to generate the cross-validation parameter set for Fir Tree WWTP, the outputs from the top one hundred model runs for all WWTPs, except Fir Tree, were used. Thus, for each WWTP, fifteen hundred model runs were provided from which a representative parameter set could be generated to simulate fifteen hundred different flow profiles. From these simulations, the median flow rate at each timestep was used to generate new diurnal fluxes, which then were compared with the relevant observed flows using NSE. Table 9 shows the NSE for the local and cross-validated, representative model.

Site	**NSE Cross-validation**	**NSE Site-specific**
Barrasford WWTP	0.87	0.94
Blanchland WWTP	0.13	0.9
Butteryhaugh WWTP	0.75	0.93
Carlton WWTP	0.54	0.9
Fir Tree WWTP	0.41	0.92
Glanton WWTP	0.78	0.89
Holy Island WWTP	0.65	0.88
Ingleby Greenhow WWTP	0.64	0.77
Powburn WWTP	0.02	0.94
Romaldkirk WWTP	0.41	0.82
Rookhope WWTP	0.72	0.89
Scots Gap WWTP	0.65	0.91
Wall WWTP	0.52	0.92
Whittingham WWTP	0.7	0.93
Whorlton WWTP	0.34	0.8
Winston WWTP	0.34	0.78

Table 9 - Results of cross-validation and comparison with site-specific model performance

As expected, the dry-weather model performs more poorly under cross-validation compared with when site-specific parameters are used. Further, the model fails completely for some WWTPs, in particular, Blanchland WWTP and Powburn WWTP. In both cases, the model performed well when using site-specific parameters, suggesting that the characteristics of the catchments may be unique in some way. When considering the ratio of the sewer network length to the length of network hull axis (Table 5) for these WWTPs, this is not surprising. In other words, the performance of the model for these WWTPs highlights the importance of some site-specific parameters. For example, the travel time distribution was not defined for each WWTP under cross-validation conditions.

It is encouraging, however, that for 56% of the WWTPs, the NSE was >0.6 under the rigorous cross-validation scenario. With a greater number of WWTPs used to generate a representative parameter set, it could be expected that this percentage would increase because the model source terms would reflect of a broader range of possible WWTP catchments.

5.4 Discussion

Here it has been shown how a simple linear reservoir function can be used to predict the wastewater flows entering small WWTPs under dry weather conditions. An inverse approach, analogous to the GLUE philosophy, means that identifying source terms is possible even in catchments where there is little or no information. The model performs well when predicting flows generated under dry weather conditions and could be used to inform the management of small WWTPs. Further, by comparing predicted versus actual flow characteristics between WWTPs and treatment performance, it may be possible to identify factors which result in variances. For example, the poor stability of a particular WWTP might be influenced by a high rate of shock-loading under dry weather conditions. The model presented, here, could allow a rapid assessment of a large network of small WWTPs with few input parameters required.

5.4.1 Future model development

To improve the relevance and accuracy of the model presented requires additional measured flow rate data for more, small WWTPs. This would allow the generation of a parameter set that is more broadly representative small WWTPs. Additionally, more data would allow for further refinement of the exiting input parameters, especially T2, D2 and γ. A more sophisticated version of the dry-weather flow model might be achieved by identifying a greater number of flow rate ‘parcels’ (Figure 13). Observation of measured dry weather flow profiles (Figure 12), reveal that some WWTPs receive small flow peaks in addition to the two large flow peaks. The importance of this characteristic was reflected in the performance of the representative flow model (Figure 16) and should be addressed to improve the accuracy of the model for a broader range of catchment scenarios.

Addressing wet weather flows requires a different approach. However, one that is similarly simple may be useful for small WWTPs, for the reasons outlined in the introduction to this chapter. The value of being able to predict such wet weather flow patterns is in extended timeseries rather than quantifying the mean diurnal profiles. It may be possible to accurately predict a long-term hydrograph for each WWTP catchment using rainfall as an input and improving the method by which the rainfall run-off is inferred (i.e., the catchment area). By subtracting the mean dry-weather profile, created using this model, from a long-term measured flow profile, it would be possible to assess the accuracy of the model, and thus, generate a representative parameter set. Adding the mean dry-weather profile to a predicted wet weather timeseries would generate a total flow hydrograph. This would be useful for exploring scenarios which may influence the wastewater flow characteristics within a catchment. For example, the effects of climate change of rainfall events and population growth resulting in an increase in dry weather flow contributions. Further, such a model could be used to assess the impact that water efficiency targets might have on the flow rates received by a WWTP. The effects of this on the performance and stability of small WWTPs remains a critical research gap, highlighting the need for more holistic water management, even in developed economies.

5.5 Conclusion

Quantifying wastewater flows received at small WWTPs is important to understand the performance and potential ecological impact of such systems. However, there is a dearth of reliable flow data because small WWTPs are not routinely monitored. Whilst there is a growing number of flow prediction models, their use is not tailored for small WWTPs because they are overly complex and rely on large datasets which may not be available for small catchments. This work has shown how an inverse solution to solving a simple linear reservoir function can generate an accurate representation of wastewater flows under dry weather conditions. The resulting model could be used to improve understanding of treatment performance and by linking the model outputs to water quality data, it could provide information on the potential impact of discharges on receiving water courses.

The demonstrated potential here leads to the recommendation that further refinement of the dry weather flow model should be carried out and the philosophy is extended to allow short to medium-term predictions under all weather conditions. The limited number of input parameters and the potential to develop a representative model, makes the analysis particularly suitable for use at small WWTPs. Under the current form, it is recommended that a representative parameter set is used to predict flows received by small WWTPs under dry weather conditions, but with site-specific values for the travel time distribution and the total wastewater flow.

Chapter 6. Application of Flow Prediction Analysis to Assess Performance Variance Between Small Wastewater Treatment Plants

6.1 Introduction

The performance and basis of reliability of small WWTPs is poorly understood. However, data presented in Chapters 3 and 4 shows a statistically significant difference between the effluent quality discharged from small and larger systems. There are various possible reasons, but one possible explanation is the effect of influent shock-loading. In larger wastewater systems, the length of the sewer network and complex characteristics of the sewage sources means that peaking factors are typically low (Tchobanoglous et al., 2003). Whereas in small systems, a steep rising limb in diurnal profiles are often prevalent. Further, under dry weather conditions, the wastewater reaching small WWTPs will be predominantly (if not exclusively) from human sources and, therefore, is often highly concentrated. Thus, when combined with the short sewer lengths and potentially rapid travel times, a sharp peak in pollutant load can occur with consequential impact on temporal performance.

The stability of mixed microbial communities, such as those found in biological treatment systems, can be affected by sudden exposure to large quantities of carbon, nutrients or other microorganisms, including those found in wastewater (Ofiteru et al., 2010; Curtis et al., 2003). The work presented in this last research chapter tests the notion that shock loading phenomena associated with small WWTPs may be a key cause of poor performance of some small systems. To do this requires high-resolution flow rate data, which can be difficult to obtain or infer for unmonitored systems (Martin & Vanrolleghem, 2014). However, the flow analysis presented in Chapter 5 provides a unique and simple solution. Therefore, by combining results from Chapters 4, 4 and 5, relationship between influent load peaking and final effluent quality can be assessed.

In this assessment, only small WWTPs were considered because defining and calculating true dry weather flow at large WWTPs is highly complex and unique to each site and catchment. Factors including the long-term effects of infiltration, non-domestic wastewater sources, and complex sewer networks which would likely involve pumped mains or storage, for example, are less likely to dominate small wastewater systems. Furthermore, comparing the daily mean load contributions of larger and small WWTPs is meaningless without reliable, high resolution flow data for the receiving water course, which was not available for most locations considered herein.

6.2 Methods

As previously established, the stability and potential impact of small WWTPs is variable between treatment types and sizes, but also across similar systems. This may be a result of variance in flow-rate characteristics. To evaluate this, the diurnal flow rate profiles were calculated for the small WWTPs assessed in Chapters 3 and 4. Briefly, bi-monthly samples were collected from twelve WWTPs with population equivalents (PE) of less than 250 and their performance was measured in terms of the removal of abiotic and genetic pollutants. The abiotic parameters included total chemical oxygen demand (tCOD), soluble chemical oxygen demand (sCOD), ammonium-nitrogen (NH_4-N), total suspended solids (TSS) and total phosphorus (TP). The genetic faecal markers were chosen because of their common use in microbial source tracking (MST) applications and included markers targeting human-specific *Bacteroides* (HF183 and HumM2), human-specific *E. coli* (H8), total *Bacteroides* (AllBac) and total *E. coli* (RodA).

The twelve WWTPs are listed in Table 10 alongside experimental design categories, which have been used to report pollutant loads measured in the final effluents. These are the same twelve small WWTPs that were studied in Chapters 3 and 4 and all final effluent data presented in this chapter are presented and discussed in Chapter 3 (for abiotic parameters) and 4 (for molecular data). For clarity, the design categories consist of waste treatment technology (i.e., activated sludge is AS, secondary filtration is SF, rotating biological contactor is RBC, and high-performance aerated filter is HiPAF) and WWTP size as PE ranges (i.e., 50-125 and 125-250). Dry weather flow data were either derived as described in section 5.2.2, or predicted, where no measured flow data were available. Eight of the WWTPs had no flow monitoring and, therefore, flow rates under dry weather conditions were predicted using a Generalised Likelihood Uncertainty Estimation (GLUE) approach to solving a linear reservoir function, which is described in Chapter 5.

To test the effects of dry weather influent peak loading on the quality of effluent discharged from small WWTPs, new influent COD concentration data were collected from two WWTPs.

Site	Design category	PE	Network Length (m)	Length of longest axis (m)	Flow monitoring
A	125-250_SF	161	2302	539	No
B	50-125_SF	72	814	249	No
C	50-125_AS	89	2448	589	No
D	50-125_SF	110	543	330	No
E	125-250_RBC	238	2248	485	Yes
F	50-125_RBC	79	681	281	No
G	50-125_RBC	68	623	278	No
H	125-250_SF	128	964	455	No
I	125-250_HiPaf	199	3046	751	Yes
J	50-125_AS	88	3404	1084	No
K	125-250_RBC	262	2932	677	Yes
L	125-250_HiPaf	188	1948	603	Yes

Table 10 - List of small WWTPs with key catchment characteristics.

6.2.1 Prediction of dry weather flow profiles for unmonitored WWTPs

The methodology presented in Chapter 5 was used to predict the mean diurnal flow profile for eight WWTPs under dry weather conditions. Throughout this study, dry weather has been defined as no rain radar measurement being detected in the forty-eight hours preceding a given time-step interval. For the purposes of prediction, therefore, it was assumed that the only contributions to the wastewater flux at each WWTP was derived from human sources. This is legitimate assumption for rural catchments like those in this study.

The input parameters for the linear reservoir function are shown in Table 11. The boundary conditions for the travel time distribution (τ) and the total human wastewater contribution (α) were defined specifically for each WWTP. Whereas, for the other parameters, values were derived from the representative model (described in Section 5.3.6). The upper and lower limits for τ were defined by Equation 8, using the sewer network parameters listed in Table 10. The boundary conditions for α were determined by the PE of each WWTP. One hundred thousand Monte Carlo simulations were used to calculate the human-derived wastewater flow and the subsequent flow received at each WWTP. The median wastewater flux for each time-step was chosen from the simulations and used to construct the average diurnal flow profile for each treatment plant.

Parameter	Description	Units	Value / range
α	Total human input	L	5,066 - 58,557
β	Proportion of α assigned to base flows	%	43
γ	Proportion of peak flows assign to first peak	%	51
T1	Start of first flow peak	Time	06:03
T2	Start of second flow peak	Time	18:17
D1	Duration of first flow peak	Hours	3.3
D2	Duration of second flow peak	Hours	5.6
τ	Travel time	Hours	0.13 – 4.47

Table 11 - Parameters used to predict dry weather flow profiles for the eight unmonitored WWTPs. Site specific values were used for the total human input and the travel time, according to the defined value ranges. All other parameters are derived from the generalise model, as described in chapter 5.

6.2.2 Calculation of dry-weather flow rates for monitored WWTPs

Four of the small WWTPs considered here were monitored for flow rates in accordance with regulatory requirements due to the ecological sensitivity of the watercourse receiving their discharge flows. Fifteen-minute flow interval data was obtained for each treatment plant for the study period (01 December 2016 – 31 October 2017). Flows rates measured under dry weather conditions, using the previous definition; i.e., conditions were considered ‘dry’ if no rainfall had been detected in the preceding 48 hours. The mean of the flow rate measured at each time-step was used to construct the average dry-weather flow profile.

To calculate the total daily dry weather flow, and therefore, the pollutant load being discharged from each WWTP, the sum of the mean flow rate at each timestep, was calculated and converted to m^3/day.

6.2.3 Estimation of effluent pollutant loads

The mean daily dry-weather load of each parameter in the final effluent of each WWTP was estimated by calculating the mean value from a series of Monte Carlo simulations. A random uniform prior distribution of concentration values was defined with the upper and lower limits of the distribution being the maximum and minimum concentrations measured in the final effluent during the study period. One thousand simulations were generated by multiplying the mean daily flow with each value of the prior distribution. The mean of the simulated loads for each parameter was used to define the indicative load contribution of each WWTP to the receiving watercourse. One-way ANOVA was used to test the significance between the simulated loads of pollutants that were discharged from different categories of WWTP.

6.2.4 Testing the effects of shock loading

Having previously confirmed a significant difference between the operating stability of small and larger systems, the effect of the load peaking phenomena on different types of small WWTPs was tested. The average performance was defined as the mean concentration of tCOD measured between December 2016 and October 2017 as previously described. Load peaking was defined as the difference between the maximum load of tCOD in influent and the mean diurnal load of tCOD in the influent.

To calculate the diurnal profile of tCOD concentration received by small WWTPs, discrete samples were collected each hour over a twenty-four-hour period at two small WWTPs (henceforth referred to as WWTP 1 and WWTP 2), during February 2018. The two small WWTPs (P.E. 72 and 128) were selected due to their proximity to Newcastle University and because their performance in terms of tCOD removal was broadly representative of the other small WWTPs assessed in Chapters 3 and 4. Under dry weather conditions, ISCO 6712 Portable automatic samplers (Teledyne ISCO, USA) were positioned and programmed to collect 1 L of influent wastewater at hourly intervals. The samples were transported to Newcastle University and analysed for tCOD using colorimetric kits (Merck, Germany) in accordance with the Standard Methods for Examination of Water and Wastewater (APHA, 2009). This provided tCOD concentration values for each hour.

The difference between the concentrations measured at each time-step and the mean measured tCOD concentration was calculated for WWTP 1 and WWTP 2. Assuming that the profiles were representative of other small WWTPs of similar size within the locality, the mean of each time-step value across the two WWTPs was used to approximate the relative change in influent tCOD concentration by time for the other small WWTPs in this study. The mean concentration of tCOD measured in the influent between December 2016 and October 2017 for each site, was used as the baseline from which the hourly concentrations were inferred.

The inferred concentration for each time-step was multiplied by the dry weather flow rate, either predicted using the flow prediction method described in Chapter 5 or measured, where monitoring data were available. The generation of the measured dry-weather flow rates is described in section 5.2.2. This produced an hourly load flow profile for each WWTP plant, where the time-step flow of influent wastewater was defined as the sum of the flow rates measured or modelled at four preceding time-steps (i.e., fifteen-minute intervals for one hour).

Thus, the load peaking of tCOD for each WWTP was defined by Equation 10.

$$Lp = \frac{\max X}{\bar{X}} \qquad (10)$$

Where X is the load of tCOD recorded at each time-step and is defined by Equation 11, where Qt is the flow at time-step t, C is the mean concentration of tCOD and therefore, dC is the difference between the concentration of tCOD at timestep t and C.

$$X = Qt * (dC * \overline{C}\,) \qquad (11)$$

The larger WWTPs that were assessed in Chapters 3 and 4 have not been considered here because determining an accurate dry weather flow rate is complex for larger WWTP and beyond the scope of this study.

6.3 Results & Discussion

6.3.1 Dry weather diurnal flow profiles

Dry weather flow profiles were determined for all small WWTPs (A – L). For the unmonitored WWTPs, a linear reservoir function was solved using an inverse approach to develop the profiles. For monitored WWTPs, the diurnal profile was derived from flow rates recorded over the study period. Mean diurnal profiles are shown in Figure 17. For the unmonitored sites, the flow profiles are remarkably similar; however, there are differences. For example, the peak flow for WWTP B occurs at 08:15, whereas for WWTP J, the peak flow occurs at 08:30. However, similarities between the profiles might be explained by three things: 1) the use of representative values for most of the input parameters, 2) the small range of network length parameters resulting is a small difference between τ values of different WWTPs (see Table 10), and 3) the coarse temporal resolution of the model which at fifteen-minute intervals might not detect small changes in flow profile characteristics.

Whilst there is a clear opportunity to further refine the flow model, it is encouraging that the predicted profiles broadly reflect those from the monitored WWTPs (E, I, K and L). With the exception of the fluctuations present in the profile of WWTP I and WWTP L, which have been previously discussed (see Section 5.2.2), the theory underpinning the model appears to be appropriate. Furthermore, the magnitude of the flow rates corresponds well to the PE of each WWTP (Table 10).

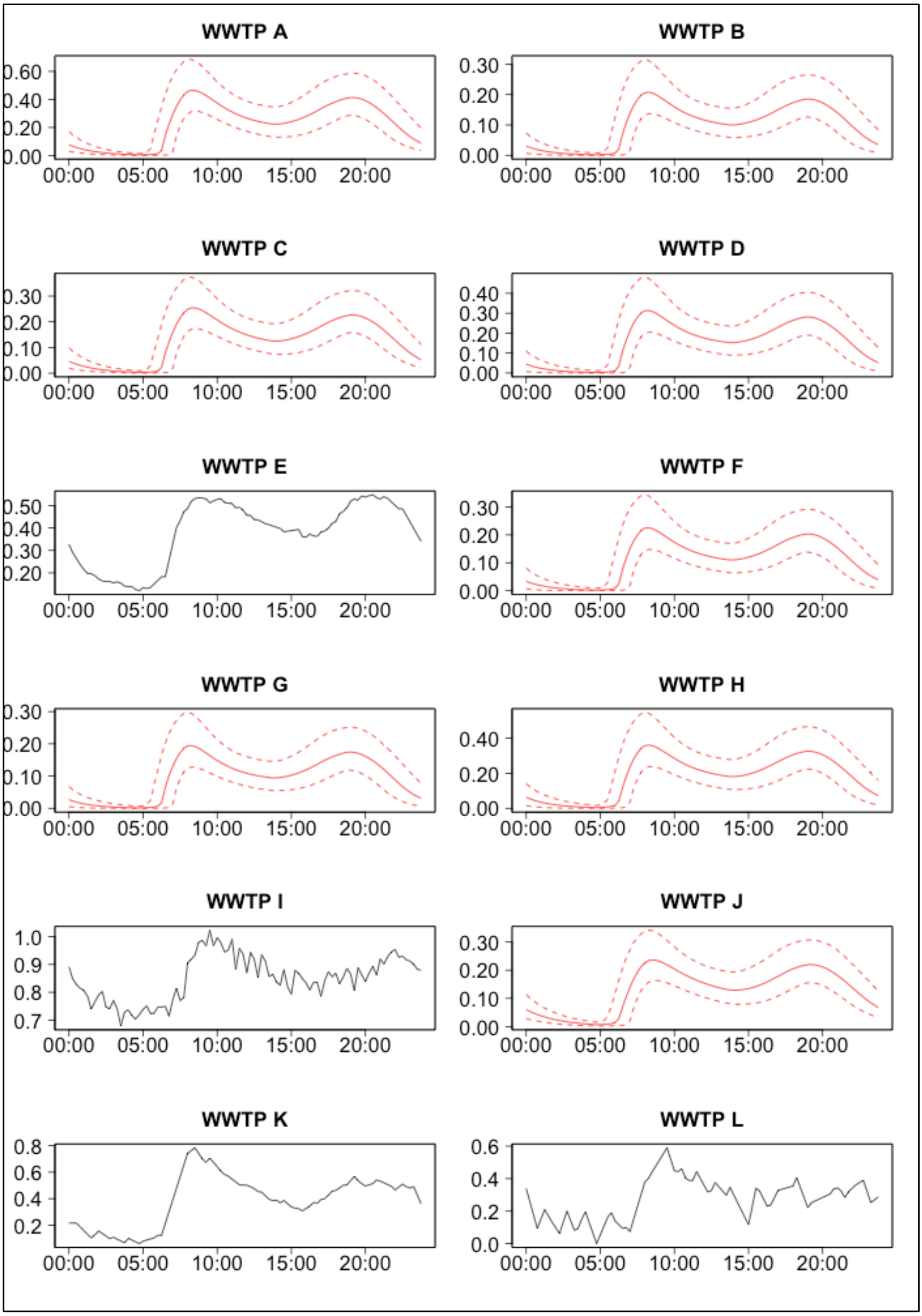


Figure 17 - Mean diurnal dry weather flow profiles (L/s) for the twelve small WWTPs. Predicted flows are shown in red with the solid red lines denoting the median flow and dashed lines showing the upper and lower quartiles. The mean diurnal dry weather flow profiles for the monitored WWTPs are shown in black.

6.3.2 Effluent pollutant loads

The most probable mean daily effluent pollutant loads were calculated for each type of WWTP and are shown in Figure 18. There is a significant difference (ANOVA, $p < 0.01$) between loads discharged from different types of WWTP for all parameters, which is consistent with differences reported in Chapter 3. The largest load of tCOD discharged per day is for the smallest trickling filter systems (50-125_SF), which concurs the findings of Chapter 3. Whereas, the type of WWTP with the lowest contribution of abiotic pollutants per day were the package plants (RBC and HiPAF), with the exception of sCOD being discharged from RBCs with PE between 125 and 250.

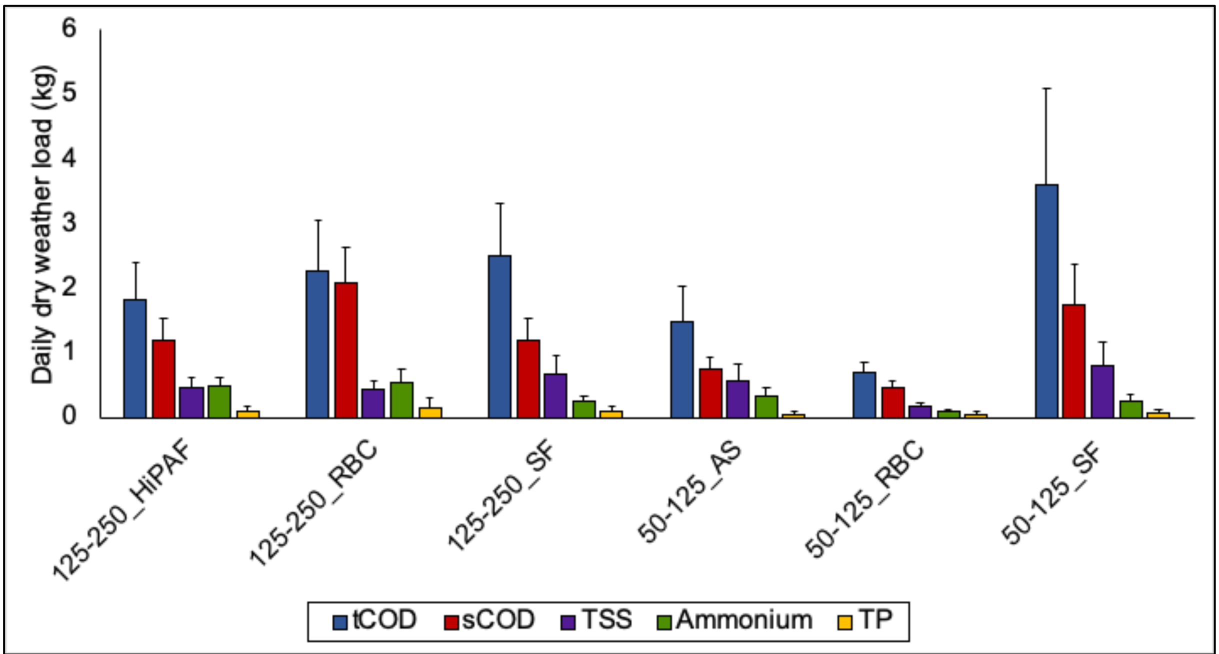


Figure 18 - Mean daily dry-weather loads of abiotic pollutants estimated in final effluent discharges from different categories of small WWTP. HiPAF is a high-performance aerated filter, RBC is rotating biological contactor, AS is activated sludge, and SF is secondary filtration. Numbers in WWTP categories denote PE.

The load of nutrients, specifically TP, is of particular interest for catchment management purposes. The commonly held perception that small WWTPs have little impact on in-river nutrient loads might be relevant at some localised scales (i.e., upstream and downstream of a single discharge point), but it has been shown that in order to fully understand the effects of pollution sources on water quality, a catchment must be viewed in its entirety (Milledge et al., 2018). Thus, the role of small WWTPs as contributors of nutrient pollution may be more important than previously thought, especially in rural catchments where there could be a high number of small communities.

For completeness, the load of the genetic faecal markers being discharged from each WWTP was calculated in the same way as the abiotic parameters (see Figure 19). The trend amongst the small WWTPs is less obvious for the genetic faecal marker loads. For example, there is a significant difference in human-specific *E. coli* (H8) loads across all WWTP types (ANOVA, $p < 0.05$), but not for any other parameters. This is likely due to seasonal variance in sunlight and temperature impacting on the concentration of genetic faecal markers in the effluents. This is discussed in detail in Chapter 4 and further supports the need for additional work on the use of genetic faecal markers for aiding the management of WWTPs across seasons in temperate climates.

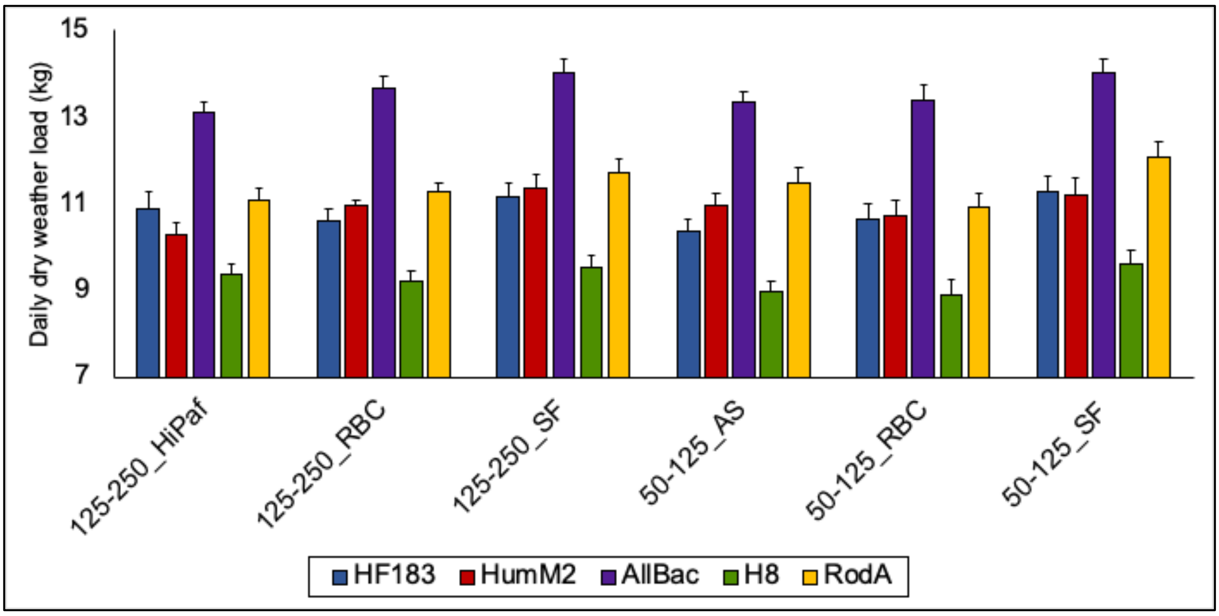


Figure 19 - Mean daily loads of genetic faecal markers estimated in final effluent discharges for different categories of small WWTP using Monte Carlo simulations. HiPAF is a high-performance aerated filter, RBC is rotating biological contactor, AS is activated sludge, and SF is secondary filtration. Numbers in WWTP categories denote PE.

However, these results provide valuable and new data on the probable contribution of faecal loads to receiving waters from small WWTPs. The highest mean loads of human-specific *Bacteroides* (HF183 and HumM2), H8, and total *Bacteroides* and *E. coli* (AllBac, RodA) were from the smallest category of trickling filter (50-125_SF). This is not surprising since this category of WWTP discharged the worst quality effluent for other performance parameters. This type of information might be useful when applying pollution source tracking techniques to upper catchments where there might be numerous small WWTPs discharging into surface waters.

6.3.3 Relationship between load peaking and effluent quality

The distance from the mean of the influent tCOD concentration, recorded under dry-weather conditions, at each time-step was inferred from samples collected at hourly intervals from two representative, small WWTPs (see section 6.2.4). The greatest difference occurred at 13:00 (Figure 20) which might appear surprising because mid-day is not typical for peak flows under dry weather conditions. However, peak flows do not necessarily imply peak pollutant concentrations because at times of peak flow (e.g., early morning), flow contributions will include grey water, whereas this may not be true later in the day. Further, the difference may be a result of 'back-ground' infiltration diluting the influent concentrations or due to non-domestic sources in the catchment, such as schools, office blocks, or cafes. The minimum tCOD concentration occurred at 03:00.

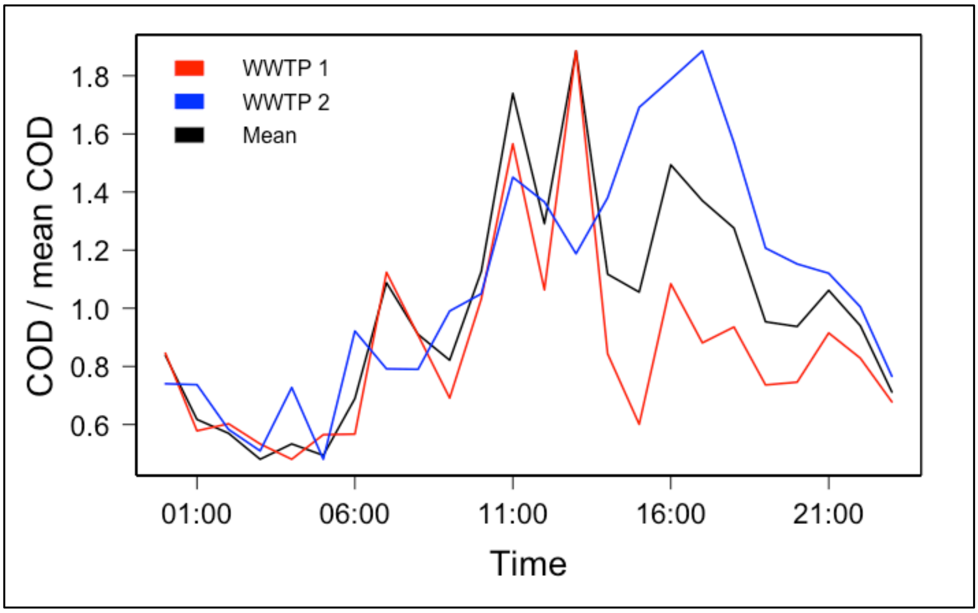


Figure 20 - Diurnal profile of influent tCOD concentration at hourly intervals as a ratio of the mean influent tCOD concentration recorded over a twenty-four-hour period. Samples were collected under dry-weather conditions. Black line is the mean, the red and blue lines are the individual WWTPs.

Finally, because the concentration profile does not follow the flow rate profile, the need to consider pollutant loads and not just concentrations over a diurnal period becomes evident. Thus, the need for reliable, high resolution flow rate data is paramount.

It is not possible to describe a general relationship between influent tCOD peaking factor under dry weather conditions and final effluent tCOD concentration, using the data provided here (Figure 21). However, this does not necessarily mean that one does not exist. Rather, it is likely a reflection of two important things:

1) The limited number of data points. Across the twelve small WWTPs, a positive non-linear relationship may be present. However, any fitted model would be strongly influenced by any of the individual datapoints, thus causing the model to overfit. Equivalent data are required for more, small WWTPs to explore the theory of dry-weather influent load peaking.
2) Higher resolution data are required to capture the true peak flows, and therefore, the peak loading. The conclusions of Chapter 5 identified the need for a higher resolution influent flow prediction model. This may also be true for measurements of COD concentration. In other words, it may only be possible to determine the effects of influent load peaking on final effluent quality, if rapid changes in influent COD load can be detected.

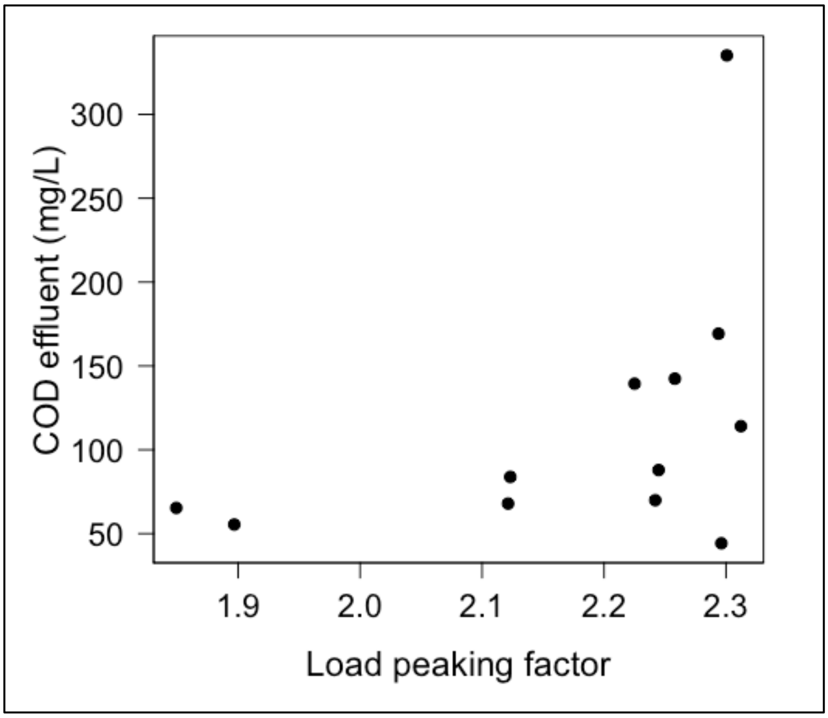


Figure 21 - Relationship between the influent tCOD load peaking factor and the mean concentration of tCOD in the final effluent of the twelve small WWTPs.

This simple analysis fails to conclusively show whether dry-weather influent load peaking is important to the performance of small WWTPs. However, it demonstrates the need for further investigation and the need for higher resolution flow rate and pollutant concentration data. More effluent quality data measured across a greater number of treatment systems are also needed.

6.4 Conclusion

This chapter shows how the use of simple flow prediction analysis can provide new and unique information about the performance of small WWTPs. Combining the model outputs with previously reported effluent quality data showed that trickling filters serving communities of between 50 and 125 PE discharged the highest load of abiotic pollutants. There was no clear relationship amongst the load of genetic faecal markers between different types of small WWTP. This is likely because of seasonal effects and the limited data.

No clear relationship could be established between influent load peaking and final effluent quality. Further investigation may be warranted but it will require higher resolution flow prediction analysis and more effluent quality data.

Chapter 7. Conclusions and Recommendations

7.1 Conclusions

The treatment performance of small WWTPs is not well understood and their potential ecological impact may be underestimated. However, the critical role they play in ensuring sustainable wastewater and water resource management means they can no longer be neglected. The aim of this thesis, therefore, was to provide new data, understanding and analytical approaches to improve the management of existing, small WWTPs. Three main objectives were fulfilled by the work presented in Chapters 3-6.

1. Improve understanding of the effect of scale and technology type on the performance and stability of small WWTPs.
2. Evaluate the potential of genetic faecal markers for assessing small WWTPs and thereby, provide insight into the potential impact of their discharges on upper catchment water quality.
3. Evaluate the influence of wastewater flow rate characteristics on the treatment performance of small WWTPs.

The field study presented in Chapter 3 revealed a significant difference ($p < 0.05$) between the treatment performance of small WWTPs and larger WWTPs, across a range of physical and chemical metrics. The stability, as covariance, followed this trend, with strong positive correlations ($r^2 = 0.45 - 0.93$) between the mean and standard deviation of the effluent samples for most parameters. Package plants seemingly provided relatively more stable performance amongst the small WWTPs, which was expected. When considering the reliability of the small WWTPs, derived from the coefficient of variation, it became clear that differences occurred, not only between technology types but also by size. With the exception of RBCs, the smallest WWTPs (50 – 125 PE) required a lower design effluent concentration than their slightly larger counterparts to achieve a final effluent tCOD of 125 mg/L. This indication that size may be particularly important to small WWTP performance, led to the development of a machine learning-based model to predict treatment reliability. It was possible to predict reliability with a reasonable degree of accuracy (64.2%)

across all types of WWTP and 100% accuracy for secondary filtration. This type of treatment was likely easy to predict because of the relatively poor stability and thus, the low design effluent concentrations. More importantly, the model revealed that the size of the WWTP was important for predicting the reliability. It is clear that with few input parameters, the simple analysis could feasibly allow wastewater managers to predict small WWTP reliability which may be useful for prioritising operational maintenance or investment.

Whilst insightful, the analysis presented in Chapter 3 did not consider seasonal effects on treatment performance and relied on limited metrics. With growing concern for water resource scarcity, there is a need to consider alternative performance metrics, especially at smaller scales. Exploiting recent advances in microbial source tracking techniques, the use of genetic faecal markers as an alternative treatment performance metric was investigated in Chapter 4. Overall, their use further supported the differences between small and larger WWTPs, especially in summer samples. Consistent with the abiotic effluent concentrations, the abundance of the genetic faecal markers revealed differences between the smallest and slightly larger WWTPs, particularly of the same technology type. Multiple clustering analyses showed that in summer, the human markers better described the variance of the effluent quality, irrespective of the target organism. In winter, however, the variance was best described by *Bacteroides* markers which can likely be explained by the difference in effect of sunlight and temperature on *Bacteroides* compared to *E. coli.* Overwhelmingly, human-specific *Bacteroides* markers proved to be the most useful as performance metrics which may be a result of the high abundance of the organisms in the human gut. Whilst there evidently is great potential for the use of such markers in wastewater management, additional work should be carried out to determine the seasonal effects on marker deterioration, especially at small WWTPs where treatment performance may be unstable.

A barrier to the effective management of most small WWTPs is the lack of flow rate monitoring. The GLUE-inspired analysis presented in Chapter 5 provided a simple solution. By using an inverse approach to solve a single equation hydrological model, it was possible to predict diurnal dry-weather flow profiles with a high level of accuracy (NSE = 0.77 – 0.94). Encouragingly, it was possible to simplify the model to just two input variables by the generation of a representative parameter set. This approach performed well under cross-validation for most WWTPs. However, the approach could be applied to predicting flows under wet weather conditions because of the dependence on rainfall events, which are not generally diurnally consistent. The flow prediction model forms the basis of a useful tool that, with small changes, be optimised for use across networks of small, unmonitored WWTPs. It has potential to provide new, useful data to inform the management of small systems and calculate the load contributions from small wastewater discharges.

The application of the flow prediction model in Chapter 6 demonstrated how such analysis can be used to further understanding and improve the management of small WWTPs. The diurnal flow profiles under dry-weather conditions was successfully predicted for all unmonitored small WWTPs which were sampled in the studies presented in Chapters 3 and 4. Whilst encouraging that the model can be used to provide new information on small WWTP performance, its application also highlights its limitations. Specifically, the temporal resolution of the model may not be high enough for the smallest catchments. However, the approach is not without merit. Combined with the physical and chemical concentration data and faecal marker abundance data, the final effluent load contributions from the small discharges followed a similar trend to the concentration data. Specifically, the differences between the smallest WWTPs and the slightly larger WWTPs was maintained. There was no clear relationship between the influent load peaking and the final effluent quality. However, further investigation may be warranted, and it would require higher resolution flow data and more effluent quality data.

The limited regulation of small wastewater discharges in England has led to a lack of monitoring and management. The work presented in this thesis has shown how simple analytical tools can be used to inform the management of small WWTPs by providing new data, proposing new performance metrics and furthering understanding of system reliability. With growing concerns regarding water scarcity and the role that decentralised infrastructure can play in sustainable wastewater management, the work is undoubtably of global relevance. However, perhaps the most useful output from the thesis is the identification of future research opportunities, the pursuit of which could lead to ‘real-world’ application of the tools and techniques presented.

7.2 Recommendations for Future Work

This thesis has demonstrated the need to pay greater attention to small WWTPs and has provided a series of simple tools to further understand and aid the management of existing assets. To further develop work presented in this thesis, it is recommended the following directions are pursued.

7.2.1 Development of operational management tools

The assessment of WWTP reliability presented in Chapter 3 provides a useful basis for the development of prioritisation and risk management tools. The analysis should be extended to a broader range of WWTP types (size and technology categories) and the predictive model developed to prioritise sites according to their likelihood of becoming unreliable. This would help to optimise asset investment and operational maintenance on small WWTPs, the management of which may become more critical for water companies in Europe especially, as compliance with legislation such as WFD (or equivalent) becomes an ever-greater concern.

For small WWTPs, the load peaking of pollutants of concern (as assessed in Chapter 6) should be further investigated. Refinement of the flow prediction approach allowing generation of higher resolution flow data may reveal a dry-weather influent load peaking as an important influencing factor on the performance of small WWTPs.

7.2.2 Temporal and spatial assessment of genetic faecal markers as performance indicators

The study presented in Chapter 4 demonstrated the potential effects of seasonality on the effective use of genetic faecal markers as WWTP performance metrics. These effects should be tested using controlled laboratory experiments and further field work. Whilst the markers clearly demonstrated potential, if their use is restricted by temperature or sunlight availability, they are of limited value to wastewater managers. Furthermore, it is well understood that genetic faecal markers are geospatially sensitive, driven by the variation in host gut microbiomes, by location. What is not well know is how this translates to their abundance in wastewater treatment systems, including influent and final effluent wastewaters. Changes in the sensitivity of different markers across catchments and regions should be tested by comparison of abundances in different wastewaters and faecal sources.

7.2.3 Refinement of flow prediction model

The simplicity and effectiveness of the flow prediction analysis described in Chapter 5 warrants its further development. The dry-weather model should be rebuilt using higher resolution flow data so that its relevance can be extended to the smallest WWTPs. Development of a wet-weather model component is essential for adoption of the presented analysis approach. By empirically deriving the relationship between measured flow data and historical rainfall events, it should be possible to forecast flow timeseries using a similar, single equation reservoir model, as used for the dry weather model. Combining the dry and wet-weather components would provide wastewater managers with the ability to rapidly acquire high-resolution flow rate data for unmonitored WWTP. In turn, this may guide operational prioritisation or, when combined with the proposal outlined in Section 7.2.1, provide unique insight into small WWTP performance and impact.

7.2.4 Development of low impact technologies

The final research focus should be on the development of wastewater treatment technologies specifically designed for small-scale applications, and that require low or no energy, are low cost, have a small footprint and are simple to operate and maintain. Several commercial applications exist (see Section 2.3 for details) but they tend to be relatively energy intensive (e.g., aerated package plants); have large land requirement (e.g., constructed wetlands), or have a high capital and operational cost (e.g., membrane bioreactors). There is a need, in England and further afield, for small-scale WWTPs that meet the above criteria and also target the removal of contaminants of emerging regulatory concern, including, nitrates, micropollutants and antibiotic resistance genes.

References

Abbassi, B.E., Abuharb, R., Ammary, B., Almanaseer, N. & Kinsley, C. (2018) Modified septic tank: Innovative onsite wastewater treatment system. *Water (Switzerland)*. 10 (5), 1–11.

Abegglen, C., Ospelt, M. & Siegrist, H. (2008) Biological nutrient removal in a small-scale MBR treating household wastewater. *Water Research*. 42 (1–2), 338–346.

Achilli, A., Cath, T.Y., Marchand, E.A. & Childress, A.E. (2009) The forward osmosis membrane bioreactor: A low fouling alternative to MBR processes. *Desalination*. 238 (1–3), 10–21.

Van Afferden, M., Cardona, J.A., Müller, R.A., Lee, M.Y. & Subah, A. (2015) A new approach to implementing decentralized wastewater treatment concepts. *Water Science and Technology*. 72 (11), 1923–1930.

Ahmed, W., Goonetilleke, A., Powell, D. & Gardner, T. (2009) Evaluation of multiple sewage-associated Bacteroides PCR markers for sewage pollution tracking. *Water Research*. 43 (19), 4872–4877.

Ahmed, W., Hughes, B. & Harwood, V.J. (2016) Current status of marker genes of bacteroides and related taxa for identifying sewage pollution in environmental waters. *Water (Switzerland)*. 8 (6), 231.

Ahmed, W., Stewart, J., Gardner, T. & Powell, D. (2008) A real-time polymerase chain reaction assay for quantitative detection of the human-specific enterococci surface protein marker in sewage and environmental waters. *Environmental Microbiology*. 10 (12), 3255–3264.

Alex, J., Hetschel, M. & Ogurek, M. (2009) Simulation study with minimised additional data requirements to analyse control and operation of WWTP Dorsten, Germany. *Water Science and Technology*. 60 (6), 1371–1377.

Allen, K. (1929) The Biological Wheel. Sewage Disposal Bulletin No. 14 1 (5).

Alvarado, S., Guédez, M., Lué-merú, M.P., Nelson, G., Alvaro, A., Jesús, A.C. & Gyula, Z. (2008) *Arsenic removal from waters by bioremediation with the aquatic plants Water Hyacinth (Eichhornia crassipes) and Lesser Duckweed (Lemna* *minor)*.

Antonie, R.L. (2017) *Fixed Biological Surfaces - Wastewater Treatment: The Rotating Biological Contactor*. Boca Raton, USA: CRC Press.

APHA (2009) *Standard Methods for the Examination of Water and Wastewater*. Washington, USA: American Public Health Association.

Ashley, R. & Cashman, A. (2007) 'The Impacts of Change on the Long-term Future Demand for Water Sector Infrastructure.', in *Infrastructure to 2030: Telecom, Land Transport, Water and Electricity*. [Online]. Paris, France: OECD. pp. 1–355.

Atkinson, S. (2018) China shows growing interest in Fluence’s smart packaged MABR business model. *Membrane Technology*. 2018 (1), 6–7.

Barro, R.J. (1988) GOVERNMENT SPENDING IN A SIMPLE MODEL OF ENDOGENOUS GROWTH. *National Bureau of Economic Research*. (2588), 1–39.

Benedetti, L., De Baets, B., Nopens, I. & Vanrolleghem, P.A. (2010) Multi-criteria analysis of wastewater treatment plant design and control scenarios under uncertainty. *Environmental Modelling and Software*. 25 (5), 616–621.

Berland, J.-M., Cooper, P. & Faby, J.-A. (2003) *Extensive wastewater treatment processes adapted to small and medium sized communities (500 to 5,000 population equivalents)*.

Bernhard, A.E. & Field, K.G. (2000) *A PCR Assay To Discriminate Human and Ruminant Feces on the Basis of Host Differences in Bacteroides-Prevotella Genes Encoding 16S rRNA*. 66 (10), 4571–4574.

Beven, K. (2012) *Rainfall-Runoff Modelling. The Primer.* Second. Chichester, UK: Wiley-Blackwell.

Beven, K. & Binley, A. (2014) *GLUE : 20 years on*. 5918 (November 2013), 5897–5918.

Beven, K. & Binley, A. (1992) *The Future of Distributed Models: Model Calibration and Uncertainty Prediction*. 6279–298.

Bichai, F. & Smeets, P.W.M.H. (2013) Using QMRA-based regulation as a water quality management tool in the water security challenge: Experience from the netherlands and australia. *Water Research*. 47 (20), 7315–7326.

BIOKUBE (2019) *Reference Countries: BioKubes has more than 7000 systems installed globally*. [Online] [online]. Available from: https://www.biokube.com/index.php/where-are-the-biokubes-installed (Accessed 22 May 2019).

Bivand, R. & Lewin-Koh, N. (2019) *maptools: Tools for Handling Spatial Objects. R Package version 0.9-5.*

BLUEWATER BIO (2019) *CFIC*. [Online] [online]. Available from: https://www.bluewaterbio.com/biowater/ (Accessed 22 May 2019).

Boehm, A.B., De Werfhorst, L.C. Van, Griffith, J.F., Holden, P.A., Jay, J.A., Shanks, O.C., Wang, D. & Weisberg, S.B. (2013) Performance of forty-one microbial source tracking methods: A twenty-seven lab evaluation study. *Water Research*. 47, 6812–6828.

Boelee, N.C., Temmink, H., Janssen, M., Buisman, C.J.N. & Wijffels, R.H. (2011) Nitrogen and phosphorus removal from municipal wastewater effluent using microalgal biofilms. *Water Research*. 45 (18), 5925–5933.

Borsuk, M.E., Maurer, M., Lienert, J. & Larsen, T.A. (2008) Charting a path for innovative toilet technology using multicriteria decision analysis. *Environmental Science and Technology*. 42 (6), 1855–1862.

Bott, C. & Parker, D.S. (2010) '*WEF/WERF Study Quantifying Nutrient Removal Technology Performance.*', in [Online]. Alexandria, VA, USA: Water Environment Research Foundation. p.

Braun, J., Mercier, J., Guillocheau, F. & Robin, C. (2016) Journal of Geophysical Research : Earth Surface A simple model for regolith formation by chemical weathering. *Journal of Geophysical Research : Earth Surface*. 121 (11), 2140–2171.

Brieman, L. (2001) Random Forestst. *Machine Learning*. 455–32.

Brown, L.E., Mitchell, G., Holden, J., Folkard, A., Wright, N., Beharry-Borg, N., Berry, G., Brierley, B., Chapman, P., Clarke, S.J., Cotton, L., Dobson, M., Dollar, E., Fletcher, M., Foster, J., Hanlon, A., Hildon, S., Hiley, P., Hillis, P., et al. (2010) Priority water research questions as determined by UK practitioners and policy makers. *Science of the Total Environment*. 409 (2), 256–266.

Brown, M.R., Camézuli, S., Davenport, R.J., Petelenz-Kurdziel, E., Øvreås, L. & Curtis, T.P. (2015) Flow cytometric quantification of viruses in activated sludge. *Water Research*. 68, 414–422.

BSI (2017) *BS EN 752:2017. Drain and sewer systems outside buildings. Sewer system management.*

Bunce, J.T., Ndam, E., Ofiteru, I.D., Moore, A. & Graham, D.W. (2018) A review of phosphorus removal technologies and their applicability to small-scale domestic wastewater treatment systems. *Frontiers in Environmental Science*. 6 (FEB), .

Bundy, C.A., Wu, D., Jong, M.-C., Edwards, S.R., Ahammad, Z.S. & Graham, D.W. (2017) Enhanced denitrification in Downflow Hanging Sponge reactors for decentralised domestic wastewater treatment. *Bioresource Technology*. 226, 1–8.

Butler, D. (1993) The Influence of Dwelling Occupancy and Day of the Week on Domestic Appliance Wastewater Discharges. *Building and Environment*. 28 (I), 73–79.

Butterworth, E., Dotro, G., Jones, M., Richards, A., Onunkwo, P., Narroway, Y. & Jefferson, B. (2013) Effect of artificial aeration on tertiary nitrification in a full-scale subsurface horizontal flow constructed wetland. *Ecological Engineering*. 54, 236–244.

Cao, Y., Sivaganesan, M., Kelty, C.A., Wang, D., Boehm, A.B., Griffith, J.F., Weisberg, S.B. & Shanks, O.C. (2018) A human fecal contamination score for ranking recreational sites using the HF183/BacR287 quantitative real-time PCR method. *Water Research*. 128, 148–156.

Capodaglio, A. (2017) Integrated, Decentralized Wastewater Management for Resource Recovery in Rural and Peri-Urban Areas. *Resources*. 6 (2), 22.

Capodaglio, A.G., Callegari, A., Cecconet, D. & Molognoni, D. (2017) Sustainability of decentralized wastewater treatment technologies. *Water Practice and Technology*. 12 (2), 463–477.

Casey, E., Glennon, B. & Hamer, G. (1999) Review of membrane aerated biofilm reactors. *Resources, Conservation and Recylcing*. 27 (December 1998), 203–215.

Castellar, J.A.., Arias, C.A., Carvalho, P., Rysulova, M. & Pérez, G. (2018) “ WETWALL ” — an innovative design concept for the treatment of wastewater at an urban scale. *Desalination and Water Treatment*. 109, 205–220.

CC Water (2019) *How much water do you use?* [Online] [online]. Available from: https://www.ccwater.org.uk/households/using-water-wisely/averagewateruse/ (Accessed 1 May 2019).

CEN (2005) *2005 European Standard EN 12566-3 ICS 13.060.30, Small wastewater treatment systems for up to 50 PT – Part 3: Packaged and/or site assembled domestic wastewater treatment plants*.

Champely, S. (2018) *pwr: Basic Functions for Power Analysis. R Package version 1.2-2*.

Chase, E., Hunting, J., Staley, C. & Harwood, V.J. (2012) Microbial source tracking to identify human and ruminant sources of faecal pollution in an ephemeral Florida river. *Journal of Applied Microbiology*. 113 (6), 1396–1406.

Chen, W., Huang, G. & Zhang, H. (2017) *Urban stormwater inundation simulation based on SWMM and diffusive overland flow*. 3392–3404.

Cheng, Z., Li, X., Bai, Y. & Li, C. (2018) Multi-Scale Fuzzy Inference System for Influent Characteristic Prediction of Wastewater Treatment. *Clean - Soil, Air, Water*. 46 (7), .

Chern, E.C., Siefring, S., Paar, J., Doolittle, M. & Haugland, R.A. (2011) Comparison of quantitative PCR assays for Escherichia coli targeting ribosomal RNA and single copy genes. *Letters in Applied Microbiology*. 52, 298–306.

Choi, S.M., Oh, Y.K. & Park, C.H. (2015) The evaluation of applicability for development of advanced package system (BioKube) for sewage treatment. *Desalination and Water Treatment*. 54 (13), 3682–3688.

Chong, M.N., Ho, A.N.M., Gardner, T., Sharma, A.K. & Hood, B. (2011) 'Assessing decentralised wastewater treatment technologies: Correlating technology selection to system robustness, energy consumption and GHG emission', in *International Conference on Integrated Water Management*. [Online]. 2011 Perth, Australia.

Cohen, J. (1988) *Statistical Power Analysis for the Behavioural Sciences (2nd edition)*. Abingdon, UK: Routledge.

Colson, P., Richet, H., Desnues, C., Balique, F., Moal, V., Grob, J.J., Berbis, P., Lecoq, H., Harlé, J.R., Berland, Y. & Raoult, D. (2010) Pepper mild mottle virus, a plant virus associated with specific immune responses, fever, abdominal pains, and pruritus in humans. *PLoS ONE*. 5 (4), .

Crites, R. & Tchobanoglous, G. (1998) 'Chapter 7: Biological treatment and nutrent removal', in Kane KT, E Munson, G Haag, & George Tchobanoglous (eds.) *Small and decentralised wastewater management systems*. [Online]. USA: WCB McGraw Hill. pp. 397–526.

Culhane, A.C., Thioulouse, J., Perrière, G. & Higgins, D.G. (2005) MADE4: An R package for multivariate analysis of gene expression data. *Bioinformatics*. 21 (11), 2789–2790.

Curtis, T.P., Head, I.M. & Graham, D.W. (2003) Theoretical Engineering: Ecology for Biology. *Environmental Science & Technology*. 37 (3), 64–70.

DEFRA (2010) *Environmental Permitting (England and Wales Regulations 2010, Statutory Instrument No. 675*.

DELPHIN (2019) *DELPHIN cube: Compact biological wastewater treatment plants wurg fixed-bed technology*. [Online]

Deng, L., Guo, W., Ngo, H.H., Zhang, H., Wang, J., Li, J., Xia, S. & Wu, Y. (2016) Biofouling and control approaches in membrane bioreactors. *Bioresource Technology*. 221, 656–665.

Devisscher, M., Ciacci, G., Fé, L., Benedetti, L., Bixio, D., Thoeye, C., De Gueldre, G., Marsili-Libelli, S. & Vanrolleghem, P.A. (2006) Estimating costs and benefits of advanced control for wastewater treatment plants – the MAgIC methodology. *Water Science and Technology*. 53 (4–5), 215–223.

DHI (2019) *MIKE URBAN+*. [Online] [online]. Available from: https://www.mikepoweredbydhi.com/products/mike-urban-plus (Accessed 23 May 2019).

EA (2018a) *General binding rules: small sewage discharge to a surface water*. [Online] [online]. Available from: https://www.gov.uk/guidance/general-binding-rules-small-sewage-discharge-to-a-surface-water (Accessed 22 May 2019).

EA (2014) *Minimum Requirements for the Self-Monitoring of Effluent Flow*. (February).

EA (2018b) *Water companies: operator self monitoring (OSM) environmental permits*. [Online] [online]. Available from: https://www.gov.uk/government/publications/water-companies-operator-self-monitoring-osm-environmental-permits/water-companies-operator-self-monitoring-osm-environmental-permits (Accessed 22 May 2019).

EC (1991) Council Directive concerning urban waste water treatment. *Official Journal of the European Communities*. 135, 40–52.

EC (2000) Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. *Official Journal of the European Parliament*. L327 (September 1996), 1–82.

Eggimann, S., Mutzner, L., Wani, O., Schneider, M.Y., Spuhler, D., Moy De Vitry, M., Beutler, P. & Maurer, M. (2017) The Potential of Knowing More: A Review of Data-Driven Urban Water Management. *Environmental Science and Technology*. 51 (5), 2538–2553.

Eggimann, S., Truffer, B., Feldmann, U. & Maurer, M. (2018) Screening European market potentials for small modular wastewater treatment systems – an inroad to sustainability transitions in urban water management? *Land Use Policy*. 78

(August 2017), 711–725.

El-shafai, S.A., El-gohary, F.A. & Nasr, F.A. (2007) *Nutrient recovery from domestic wastewater using a UASB-duckweed ponds system*. 98, 798–807.

Environment Agency (2015) *Update to the river basin management plans in England. National evidence and data report*. (December).

Erbe, V., Frehmann, T., Geiger, W.F., Krebs, P., Londong, J., Rosenwinkel, K.-H. & Seggelke, K. (2002) Integrated modelling as an analytical and optimisation tool for urban watershed management Integrated modelling as an analytical and optimisation tool for urban watershed management. *Water Science and Technology*. 46 (6–7), 141–150.

Farmer, D., Sivapalan, M. & Jothityangkoon, C. (2003) Climate, soil, and vegetation controls upon the variability of water balance in temperate and semiarid landscapes: Downward approach to water balance analysis. *Water Resources Research*. 39 (2), 1–21.

Fluence (2019) *MABR Wastewater Treatment*. [Online] [online]. Available from: https://www.fluencecorp.com/mabr/ (Accessed 22 May 2019).

Fonder, N. & Headley, T. (2016) *Chapter 15 Systematic Classification , Nomenclature and Reporting for Constructed Treatment Wetlands*. (August), .

Fowdar, H.S., Hatt, B.E., Breen, P., Cook, P.L.M. & Deletic, A. (2017) Designing living walls for greywater treatment. *Water Research*. 110, 218–232.

Friedler, E., Butler, D. & Brown, D. (1996) Domestic WC Usage Patters. *Building and Environment*. 31 (4), 385–392.

Friedman, A.A., Robbins, L.E. & Woods, R.C. (1979) Effect of Disk Rotational Speed on Biological Contactor Efficiency. *Journal (Water Pollution Control Federation)*. 51 (11), 2678–2690.

Fronteau, C., Bauwens, W. & Vanrolleghm, P.A. (1997) Integrated Modelling: Comparison of State Varibles Processes and Parameters in Sewers and Wastewater Treatment Plant Models. *Water Science & Technology*. 36 (5), 373–380.

Gawler, A.H., Beecher, J.E., Brandão, J., Carroll, N.M., Falcão, L., Gourmelon, M., Masterson, B., Nunes, B., Porter, J., Rincé, A., Rodrigues, R., Thorp, M., Martin Walters, J. & Meijer, W.G. (2007) Validation of host-specific Bacteriodales 16S rRNA genes as markers to determine the origin of faecal pollution in Atlantic Rim countries of the European Union. *Water Research*. 41 (16), 3780–3784.

Gernaey, K. V., Flores-Alsina, X., Rosen, C., Benedetti, L. & Jeppsson, U. (2011) Dynamic influent pollutant disturbance scenario generation using a phenomenological modelling approach. *Environmental Modelling and Software*. 26 (11), 1255–1267.

Gernaey, K. V., Rosen, C., Benedetti, L. & Jeppsson, U. (2005) Phenomenological Modeling of Wastewater Treatment Plant Influent Disturbance Scenarios. *10th International Conference on Urban Drainage, Copenhagen/Denmark*. 1 (1), 21–26.

Gernaey, K. V, Rosen, C. & Jeppsson, U. (2006) WWTP dynamic disturbance modelling–an essential module for long-term benchmarking development. *Water Science and Technology*. 53 (4–5), 225–234.

Gevaert, V., Verdonck, F., Benedetti, L., De Keyser, W. & De Baets, B. (2009) Evaluating the usefulness of dynamic pollutant fate models for implementing the EU Water Framework Directive. *Chemosphere*. 76 (1), 27–35.

Gikas, P. & Tchobanoglous, G. (2009) The role of satellite and decentralized strategies in water resources management. *Journal of Environmental Management*. 90 (1), 144–152.

Gomi, R., Matsuda, T., Matsui, Y. & Yoneda, M. (2014) Fecal Source Tracking in Water by Next-Generation Sequencing Technologies Using Host-Specific *Escherichia coli* Genetic Markers. *Environmental Science & Technology*. 48 (16), 9616–9623.

Gosling, S.N. & Arnell, N.W. (2016) A global assessment of the impact of climate change on water scarcity. *Climatic Change*. 134 (3), 371–385.

Graham, D., Giesen, M., Bunce, J., Graham, D.W., Giesen, M.J. & Bunce, J.T. (2019) Strategic Approach for Prioritising Local and Regional Sanitation

Interventions for Reducing Global Antibiotic Resistance. *Water*. 11 (1), 27.

Grau, P. (1996) Low cost wastewater treatment. *Water Science and Technology*. 33 (8), 39–46.

Greaves, F.E., Thorp, B. & Critchley, R.F. (1990) Operational Performance of Package Sewage Treatment Plants in North West England. *Water Science and Technology*. 22 (3–4), 25–32.

Green, H.C., Haugland, R.A., Varma, M., Millen, H.T., Borchardt, M.A., Field, K.G., Walters, W.A., Knight, R., Sivaganesan, M., Kelty, C.A. & Shanks, O.C. (2014) Improved HF183 quantitative real-time PCR assay for characterization of human fecal pollution in ambient surface water samples. *Applied and Environmental Microbiology*. 80 (10), 3086–3094.

Green, H.C., Shanks, O.C., Sivaganesan, M., Haugland, R.A. & Field, K.G. (2011) Differential decay of human faecal Bacteroides in marine and freshwater. *Environmental Microbiology*. 13 (12), 3235–3249.

Gurney (2019) *Aero-Fac Wastewater Treatment System*. [Online] [online]. Available from: https://gurneyenvironmental.com/index.php/aero-fac-wastewater-technology (Accessed 22 May 2019).

Gustafsson, L.-G., Lumley, D.J., Lindeborg, C. & Haraldsson, J. (1993) Integrating a Catchment Simulator into Wastewater Treatment Plant Operation. *Water Science and Technology*. 28 (11–12), 45–54.

Gutterer, B., Sasse, L., Panzerbieter, T. & Reckerzugel, T. (2009) *Decentralised Wastewater Treatment Systems (DEWATS) and Sanitation in Developing Countries*. 49 (0).

Harwood, V.J., Levine, A.D., Scott, T.M., Chivukula, V., Lukasik, J., Farrah, S.R. & Rose, J.B. (2005) Validity of the indicator organism paradigm for pathogen reduction in reclaimed water and public health protection. *Applied and environmental microbiology*. 71 (6), 63–70.

Harwood, V.J., Staley, C., Badgley, B.D., Borges, K. & Korajkic, A. (2014) Microbial source tracking markers for detection of fecal contamination in environmental waters: Relationships between pathogens and human health outcomes. *FEMS*

Microbiology Reviews. 38 (1), 1–40.

Hassard, F., Biddle, J., Cartmell, E., Jefferson, B., Tyrrel, S. & Stephenson, T. (2015) Rotating biological contactors for wastewater treatment - A review. *Process Safety and Environmental Protection*. 94 (C), 285–306.

Hassard, F., Cartmell, E., Biddle, J. & Stephenson, T. (2014) Performance of permeable media rotating reactors used for pretreatment of wastewaters. *Water Science and Technology*. 69 (9), 1926–1931.

Hastie, T., Tibshirani, R. & Friedman, J. (2008) *The Elements of Statistical Learning*. Stanford: Springer.

Heinonen-tanski, H. & Matikka, V. (2017) *Chemical and Microbiological Quality of Effluents from Different On-Site Wastewater Treatment*.

Hernebring, C., Jönsson, L.-E., Thorén, U.-B. & Møller, A. (2002) Dynamic online sewer modelling in Helsingborg. *Water Science and Technology*. 45 (4–5), 429–436.

Hijmans, R.J. (2019) *raster: Geographic Data Analysis and Modeling. R package version 2.8-19.*

Hiras, D.N., Manariotis, I.D. & Grigoropoulos, S.G. (2004) Organic and nitrogen removal in a two-stage rotating biological contactor treating municipal wastewater. *Bioresource Technology*. 93 (1), 91–98.

Ho, G. (2005) Technology for sustainability: the role of onsite, small and community scale technology. *Water Science and Technology*. 51 (10), 15–20.

Ho, T.K. (1995) Random decision forests. *Proceedings of the International Conference on Document Analysis and Recognition, ICDAR*. 1278–282.

Hong, S.-W., Choi, Y.-S., Kim, S.-J. & Kwon, G. (2005) Pilot-testing an alternative on-site wastewater treatment system for small communities and its automatic control. *Water Science and Technology*. 51 (10), 101–108.

Horan, N.J., Salih, A. & Walkinshaw, T. (2006) Wind-aerated lagoons for sustainable treatment of wastewaters from small communities. *Water and Environment Journal*. 20 (4), 265–270.

Hörman, A., Rimhanen-finne, R., Maunula, L., Bonsdorff, C. Von & Torvela, N. (2004) Indicator organisms in surface water in southwestern. *Applied and Environmental Microbiology*. 70 (1), 87–95.

Hothorn, T. & Everitt, B.S. (2014) *A Handbook of Statistical Analysis Using R*. London, UK: Chapman and Hall.

Hughes, B., Beale, D.J., Dennis, P.G., Cook, S. & Ahmed, W. (2017) Cross-Comparison of Human Wastewater-Associated Molecular Markers in Relation to Fecal Indicator Bacteria and Enteric Viruses in Recreational Beach Waters. *Applied and environmental microbiology*. 83 (8), .

Huijbers, P.M.C., Blaak, H., De Jong, M.C.M., Graat, E.A.M., Vandenbroucke-Grauls, C.M.J.E. & De Roda Husman, A.M. (2015) Role of the Environment in the Transmission of Antimicrobial Resistance to Humans: A Review. *Environmental Science and Technology*. 49 (20), 11993–12004.

Ilyas, H. & Masih, I. (2017) The performance of the intensi fi ed constructed wetlands for organic matter and nitrogen removal : A review. *Journal of Environmental Management*. 198, 372–383.

Innovyze (2019) *Infoworks ICM*. [Online] [online]. Available from: https://www.innovyze.com/en-us/products/infoworks-icm (Accessed 23 May 2019).

Istenic, D., Bodík, I. & Bulc, T. (2015) Status of decentralised wastewater treatment systems and barriers for implementation of nature-based systems in central and eastern Europe. *Environmental Science and Pollution Research*. 22 (17), 12879–12884.

Jeppsson, U., Alex, J., Batstone, D.J., Benedetti, L., Comas, J., Copp, J.B., Corominas, L., Flores-Alsina, X., Gernaey, K. V, Nopens, I., Pons, M.-N., Rodríguez-Roda, I., Rosen, C., Steyer, J.-P., Vanrolleghem, P.A., Volcke, E.I.P. & Vrecko, D. (2013) Benchmark simulation models, quo vadis? *Water Science and Technology*. 68 (1), 1–15.

Jonasson, O.J. & Kandasamy, J. (2017) Decentralised water reuse in Sydney, Australia: drivers for implementation and energy consumption. *Journal of*

Environmental Engineering and Science. 13 (1), 2–7.

Jong, M.-C., Su, J.-Q., Bunce, J.T., Harwood, C.R., Snape, J.R., Zhu, Y.-G. & Graham, D.W. (2018) Co-optimization of sponge-core bioreactors for removing total nitrogen and antibiotic resistance genes from domestic wastewater. *Science of the Total Environment*. 634.

Jorsaraei, A., Gougol, M. & Van Lier, J.B. (2014) A cost effective method for decentralized sewage treatment. *Process Safety and Environmental Protection*. 92 (6), 815–821.

Judd, S. (2008) *The status of membrane bioreactor technology*. i (January), 109–116.

Kabiri, L., Alum, A., Rock, C., McLain, J.E. & Abbaszadegan, M. (2016) A tool box strategy using Bacteroides genetic markers to differentiate human from non-human sources of fecal contamination in natural water. *Science of The Total Environment*. 572, 897-905.

Kesaano, M. & Sims, R.C. (2014) Algal biofilm based technology for wastewater treatment. *Algal Research*. 5 (1), 231–240.

Kildare, B.J., Leutenegger, C.M., McSwain, B.S., Bambic, D.G., Rajal, V.B. & Wuertz, S. (2007) 16S rRNA-based assays for quantitative detection of universal, human-, cow-, and dog-specific fecal Bacteroidales: A Bayesian approach. *Water Research*. 41 (16), 3701–3715.

Kirchner, J.W. (2009) Catchments as simple dynamical systems: Catchment characterization, rainfall-runoff modeling, and doing hydrology backward. *Water Resources Research*. 45 (2), 1–34.

Kirs, M., Caffaro-Filho, R.A., Wong, M., Harwood, V.J., Moravcik, P. & Fujioka, R.S. (2016) Human-Associated Bacteroides spp. and Human Polyomaviruses as Microbial Source Tracking Markers in Hawaii. *Applied and environmental microbiology*. 82 (22), 6757–6767.

Klees, R. & Silverstein, J. (1992) Improved biological nitrification using recirculation in rotating biological contactors. *Water Science and Technology*. 26 (3–4), 545–553.

Koudelak, P. & West, S. (2008) Sewerage network modelling in Latvia, use of info works CS and storm water management model 5 in Liepaja city. *Water and Environment Journal*. 22 (2), 81–87.

Kuhn, M. (2018) *caret: Classification and Regression Training. R Package version 6.0-80*.

Kusiak, A., Verma, A. & Wei, X. (2013) A data-mining approach to predict influent quality. *Environmental Monitoring and Assessment*. 185 (3), 2197–2210.

Langergraber, G., Alex, J., Weissenbacher, N., Woerner, D., Ahnert, M., Frehmann, T., Halft, N., Hobus, I., Plattes, M., Spering, V. & Winkler, S. (2008) Generation of diurnal variation for influent data for dynamic simulation. *Water Science and Technology*. 57 (9), 1483–1486.

Langergraber, G., Pressl, A., Kretschmer, F. & Weissenbacher, N. (2018) Small wastewater treatment plants in Austria – Technologies, management and training of operators. *Ecological Engineering*. 120 (June), 164–169.

Langergraber, G. & Weissenbacher, N. (2017) Survey on number and size distribution of treatment wetlands in Austria. *Water Science and Technology*. 75 (10), 2309–2315.

Larsen, T.A., Alder, A.C., Eggen, R.I.L., Maurer, M. & Lienert, J. (2009) A new planning and design paradigm to achieve sustainable resource recovery from wastewater. *Environmental Science and Technology*. 43 (16), 6126–6130.

Layton, A., Mckay, L., Williams, D., Garrett, V., Gentry, R. & Sayler, G. (2006) Development of Bacteroides 16S rRNA gene taqman-based real-time PCR assays for estimation of total, human, and bovine fecal pollution in water. *Applied and Environmental Microbiology*. 72 (6), 4214–4224.

Lee, D.Y., Weir, S.C., Lee, H. & Trevors, J.T. (2010) Quantitative identification of fecal water pollution sources by TaqMan real-time PCR assays using Bacteroidales 16S rRNA genetic markers. *Applied Microbiology and Biotechnology*. 88 (6), 1373–1383.

Leong, J.Y.C., Oh, K.S., Poh, P.E. & Chong, M.N. (2017) Prospects of hybrid rainwater-greywater decentralised system for water recycling and reuse: A review. *Journal of Cleaner Production*. 142, 3014–3027.

Limpert, E., Stahel, W.A. & Abbt, M. (2001) Log-normal distributions across the sciences: keys and clues. *BioScience*. 51 (5), .

Madigan, M.T., Martinko, J.M., Stahl, D.A. & Clark, D.. (2012) Page 26 - useful for microscopy dye.Page 28 - DAPI stainingTEM - cell components and structureSEM - cell surfacehaploid - single copy of each geneHydrophobic - fatty acidhydrophilic - glycerol-phosphatePage 55 - Three membrane transport mechanisms exist in bacteria - simple, group translocation and ABC transport.Pgae 128 - cell counts - microscopy and flow cytometry. Really should use DAPI or fluorescent staining.Page 129 - plate counts - spread plates and pour-plates.Page 130 - diluting cell suspensions. 10-fold and 100-fold.Page 674 - biofilms. *Brock Biology of microorganisms*. D. Espinoza (ed.). San Francisco: Benjamin Cummings.

Mahmoodian, M., Carbajal, J.P., Bellos, V., Leopold, U., Schutz, G. & Clemens, F. (2018) A Hybrid Surrogate Modelling Strategy for Simplification of Detailed Urban Drainage Simulators. *Water Resources Management*. 32, 5241–5256.

Mann, A.T., Mendoza-espinosa, L. & Stephenson, T.O.M. (1999) *TECHNICAL NOTE PERFORMANCE OF FLOATING AND SUNKEN MEDIA BIOLOGICAL AERATED FILTERS UNDER UNSTEADY STATE CONDITIONS*. 33 (4), 1108–1113.

Mannina, G., Cosenza, A., Vanrolleghem, P.A. & Viviani, G. (2011) A practical protocol for calibration of nutrient removal wastewater treatment models. *Journal of Hydroinformatics*. 13 (4), 575–595.

Mara, D. (2006) Good Practice in Water and Environmental Management: Natural Wastewater Treatment. N. J. Horan (ed.). *Chartered Institute of Water and Environmental Management. London, UK*.

Marinaki, M. & Papageorgiou, M. (2005) *Optimal Real-time Control of Sewer Networks*. London, UK: Springer.

Martin, C. & Vanrolleghem, P.A. (2014) Analysing, completing, and generating influent data for WWTP modelling: A critical review. *Environmental Modelling* *and Software*. 60188–201.

Masi, F., Bresciani, R., Rizzo, A., Edathoot, A., Patwardhan, N., Panse, D. & Langergraber, G. (2016) Green walls for greywater treatment and recycling in dense urban areas: a case-study in Pune. *Journal of Water, Sanitation and Hygiene for Development*. 6 (2), 342–347.

Massoud, M.A., Tarhini, A. & Nasr, J.A. (2009) Decentralized approaches to wastewater treatment and management: Applicability in developing countries. *Journal of Environmental Management*. 90 (1), 652–659.

Maurer, M. (2009) Specific net present value: An improved method for assessing modularisation costs in water services with growing demand. *Water Research*. 43 (8), 2121–2130.

Maurer, M., Rothenberger, O. & Larsen, T.A. (2005) Decentralised wastewater treatment technologies from a national perspective: At what cost are they competitive? *Water Science and Technology: Water Supply*. 5 (6), 145–154.

May, L., Place, C., OMalley, M. & Spears, B.M. (2015) *The impact of phosphorus inputs from small discharges on designated freshwater sites. Natural England Commissioned Reports, Number 170.* (June).

Mayer, R.E., Bofill-Mas, S., Egle, L., Reischer, G.H., Schade, M., Fernandez-Cassi, X., Fuchs, W., Mach, R.L., Lindner, G., Kirschner, A., Gaisbauer, M., Piringer, H., Blaschke, A.P., Girones, R., Zessner, M., Sommer, R. & Farnleitner, A.H. (2016) Occurrence of human-associated Bacteroidetes genetic source tracking markers in raw and treated wastewater of municipal and domestic origin and comparison to standard and alternative indicators of faecal pollution. *Water Research*. 90, 265–276.

Mayer, R.E., Reischer, G.H., Ixenmaier, S.K., Derx, J., Blaschke, A.P., Ebdon, J.E., Linke, R., Egle, L., Ahmed, W., Blanch, A.R., Byamukama, D., Savill, M., Mushi, D., Cristóbal, H.A., Edge, T.A., Schade, M.A., Aslan, A., Brooks, Y.M., Sommer, R., et al. (2018) Global Distribution of Human-Associated Fecal Genetic Markers in Reference Samples from Six Continents. *Environmental Science and Technology*. 52 (9), 5076–5084.

Mayer, R.E., Vierheilig, J., Egle, L., Reischer, G.H., Saracevic, E., Mach, R.L., Kirschner, A.K.T., Zessner, M., Sommer, R. & Farnleitner, A.H. (2015) Automated sampling procedures supported by high persistence of bacterial fecal indicators and Bacteroidetes genetic microbial source tracking markers in municipal wastewater during short-term storage at 5°C. *Applied and Environmental Microbiology*. 81 (15), 5134–5143.

McCarty, P.L., Bae, J. & Kim, J. (2011) Domestic wastewater treatment as a net energy producer - can this be achieved? *Environmental science & technology*. 45 (17), 7100-7106.

McDonald, R.I., Weber, K., Padowski, J., Flörke, M., Schneider, C., Green, P.A., Gleeson, T., Eckman, S., Lehner, B., Balk, D., Boucher, T., Grill, G. & Montgomery, M. (2014) Water on an urban planet: Urbanization and the reach of urban water infrastructure. *Global Environmental Change*. 27 (1), 96–105.

McQuaig, S., Griffith, J. & Harwood, V.J. (2012) Association of fecal indicator bacteria with human viruses and microbial source tracking markers at coastal beaches impacted by nonpoint source pollution. *Applied and Environmental Microbiology*. 78 (18), 6423–6432.

McQuaig, S.M., Scott, T.M., Lukasik, J.O., Paul, J.H. & Harwood, V.J. (2009) Quantification of human polyomaviruses JC virus and BK Virus by TaqMan quantitative PCR and comparison to other water quality indicators in water and fecal samples. *Applied and Environmental Microbiology*. 75 (11), 3379–3388.

Met Office (2010) *Met Office: UK Climate - historic station data 1981-2010*. [Online] [online]. Available from: https://www.metoffice.gov.uk/public/weather/climate-historic/#?tab=climateHistoric (Accessed 1 April 2017).

Metz, C.E. (1978) Basic principles of ROC analysis. *Seminars in Nuclear Medicine*. 8 (4), 283–298.

Milledge, D.G., Gurjar, S.K., Bunce, J.T., Tare, V., Sinha, R. & Carbonneau, P.E. (2018) Population density controls on microbial pollution across the Ganga catchment. *Water Research*. 128, 82-91.

Nasr, F.A. & Mikhaeil, B. (2015) Treatment of domestic wastewater using modified septic tank. *Desalination and Water Treatment*. 56 (8), 2073–2081.

Niku, S., Schroeder, E.D. & Samaniego, F.J. (1979) Performance of activated sludge processes and reliability-based design. *Journal of Water Pollution Control*. 51 (12), 2841–2857.

Nivala, J., Abdallat, G., Aubron, T., Al-Zreiqat, I., Abbassi, B., Wu, G.-M., van Afferden, M. & Müller, R.A. (2019) Vertical flow constructed wetlands for decentralized wastewater treatment in Jordan: Optimization of total nitrogen removal. *Science of The Total Environment*. 671, 495–504.

Nivala, J., Headley, T., Wallace, S., Bernhard, K., Brix, H., van Afferden, M. & Müller, R.A. (2013) Comparative analysis of constructed wetlands: The design and construction of the ecotechnology research facility in Langenreichenbach, Germany. *Ecological Engineering*. 61, 527–543.

Noble, R.T., Lee, I.M. & Schiff, K.C. (2004) Inactivation of indicator micro-organisms from various sources of faecal contamination in seawater and freshwater. *Journal of Applied Microbiology*. 96 (3), 464–472.

Ofiteru, I.D., Lunn, M., Curtis, T.P., Wells, G.F., Criddle, C.S., Francis, C.A. & Sloan, W.T. (2010) Combined niche and neutral effects in a microbial wastewater treatment community. *Proceedings of the National Academy of Sciences*. 107 (35), 15345–15350.

Ostace, G.S., Cristea, V.M. & Agachi, P.Ş. (2011) Cost reduction of the wastewater treatment plant operation by MPC based on modified ASM1 with two-step nitrification/denitrification model. *Computers and Chemical Engineering*. 35 (11), 2469–2479.

OXYMEM (2018) *Wessex Water Installs the World 's First OxyTUBE*.

Pachepsky, Y.A., Blaustein, R.A., Whelan, G. & Shelton, D.R. (2014) Comparing temperature effects on Escherichia coli, Salmonella, and Enterococcus survival in surface waters. *Letters in Applied Microbiology*. 59 (3), 278–283.

Pai, T.Y., Wan, T.J., Hsu, S.T., Chang, T.C., Tsai, Y.P., Lin, C.Y., Su, H.C. & Yu, L.F. (2009) Using fuzzy inference system to improve neural network for predicting hospital wastewater treatment plant effluent. *Computers and Chemical*

Engineering. 33 (7), 1272–1278.

Pai, T.Y., Yang, P.Y., Wang, S.C., Lo, M.H., Chiang, C.F., Kuo, J.L., Chu, H.H., Su, H.C., Yu, L.F., Hu, H.C. & Chang, Y.H. (2011) Predicting effluent from the wastewater treatment plant of industrial park based on fuzzy network and influent quality. *Applied Mathematical Modelling*. 35 (8), 3674–3684.

Di Palma, L. & Verdone, N. (2009) The effect of disk rotational speed on oxygen transfer in rotating biological contactors. *Bioresource Technology*. 100 (3), 1467–1470.

Park, Y.-T. (2015) *Septic tank for generating methane gas*. 9969636.

Parveen, S., Portier, K.M., Robinson, K., Edmiston, L. & Tamplin, M.L. (1999) Discriminant analysis of ribotype profiles of Escherichia coli for differentiating human and nonhuman sources of fecal pollution. *Applied and Environmental Microbiology*. 65 (7), 3142–3147.

Patwardhan, A.W. (2003) Rotating biological contactors: A review. *Industrial and Engineering Chemistry Research*. 42 (10), 2035–2051.

Polprasert, C. (2004) Constructed Wetlands for Wastewater Treatment: Principles and Practices. *Wetlands Ecosystems in Asia*. 1 (04), 285–310.

Praveen, P. & Loh, K.-C. (2016) Nitrogen and Phosphorus Removal from Tertiary Wastewater in an Osmotic Membrane Photobioreactor. *Bioresource Technology*. 206, 180–187.

Pujol, R. & Lienard, A. (1989) 'Qualitative and quantitative characterization of waste water for small communities', in H. Odegaard (ed.) *International Specialized Conference on Design and Operation of Small Wastewater Treatment Plants*. [Online]. 1989 Trondheim, Norway. pp. 267–274.

QGIS Development Team (2019) *QGIS Geographical Information System. Open Source Geospatial Foundation Project*.

R Core (2018) *R: A language and environment for statistical computing. R Foundation for Statistical Computing*.

Reischer, G.H., Kasper, D.C., Steinborn, R., Farnleitner, A.H. & Mach, R.L. (2007) A quantitative real-time PCR assay for the highly sensitive and specific detection of human faecal influence in spring water from a large alpine catchment area. *Letters in applied microbiology*. 44 (4), 351–356.

Richards, S., Paterson, E., Withers, P.J.A. & Stutter, M. (2016) Septic tank discharges as multi-pollutant hotspots in catchments. *Science of the Total Environment*. 542, 854–863.

Roefs, I., Meulman, B., Vreeburg, J.H.G. & Spiller, M. (2017) Centralised, decentralised or hybrid sanitation systems? Economic evaluation under urban development uncertainty and phased expansion. *Water Research*. 109, 274–286.

Ronteltap, M., Maurer, M. & Gujer, W. (2007) The behaviour of pharmaceuticals and heavy metals during struvite precipitation in urine. *Water Research*. 41 (9), 1859–1868.

Sadoff, C.W., Hall, J.W., Grey, D., Aerts, J.C.J.H., Ait-Kadi, M., Brown, C., Cox, A., Dadson, S., Garrick, D., Kelman, J., McCornick, P., Ringler, C., Rosegrant, M., Whittington, D. & Wiberg, D. (2015) Securing Water, Sustaining Growth. Report of the GWP/OECD Task Force on Water Securit (70).

Sedlak, D. (2015) '*Water 4.0*', in [Online]. Connecticut, USA: Yale University Press. p. 352.

Seurinck, S., Defoirdt, T., Verstraete, W. & Siciliano, S.D. (2005) Detection and quantification of the human-specific HF183 Bacteroides 16S rRNA genetic marker with real-time PCR for assessment of human faecal pollution in freshwater. *Environmental Microbiology*. 7 (2), 249–259.

Seurinck, S., Verstraete, W. & Siciliano, S.D. (2005) Microbial source tracking for identification of fecal pollution. *Reviews in Environmental Science and Biotechnology*. 4 (1–2), 19–37.

Shanks, O.C., Kelty, C.A., Sivaganesan, M., Varma, M. & Haugland, R.A. (2009) Quantitative PCR for genetic markers of human fecal pollution. *Applied and Environmental Microbiology*. 75 (17), 5507–5513.

Shanks, O.C., White, K., Kelty, C. a, Sivaganesan, M., Blannon, J., Meckes, M. &

Varma, M. (2010) Performance of PCR Based Assays Targeting Bacteriodales Genetic Markers of Human Fecal Pollution in Sewage and Fecal Samples. *Environmental Science and Technology*. 44 (16), 6281–6288.

Shiraishi, Y., Tremmel, G., Miyano, S. & Stephens, M. (2015) A Simple Model-Based Approach to Inferring and Visualizing Cancer Mutation Signatures. *PLoS Genetics*. 11 (12), 1–21.

Siegrist, R.L. (2017) 'Treatment Using Septic Tanks', in *Decentralized Water Reclamation Engineering*. [Online]. Berlin, Germany: Springer.

Silkie, S.S. & Nelson, K.L. (2009) Concentrations of host-specific and generic fecal markers measured by quantitative PCR in raw sewage and fresh animal feces. *Water Research*. 43 (19), 4860–4871.

Singh, N.K., Kazmi, A.A. & Starkl, M. (2015) A review on full-scale decentralized wastewater treatment systems: Techno-economical approach. *Water Science and Technology*. 71 (4), 468–478.

von Spelburg, M. & Augusto de Lemos Chernicharo, C. (2006) *Biological Wastewater Treatment in Warm Climate Regions*. London, UK: IWA Publishing.

Srinivasan, S., Aslan, A., Xagoraraki, I., Alocilja, E. & Rose, J.B. (2011) Escherichia coli, enterococci, and Bacteroides thetaiotaomicron qPCR signals through wastewater and septage treatment. *Water Research*. 45 (8), 2561–2572.

Stensel, H., C., B.R., M., L.K., H., M. & K., R. (1988) Biological Aerated Filter Evaluation. *Journal of Environmental Engineering*. 114 (3), 655–671.

Talebizadeh, M., Belia, E. & Vanrolleghem, P.A. (2016) Influent generator for probabilistic modeling of nutrient removal wastewater treatment plants. *Environmental Modelling and Software*. 77, 32–49.

Tchobanoglous, G., Burton, F.. & Stensel, D. (2003) *Wastewater Engineering: Treatment and Reuse (4th Edition)*. 4th edition. New York, USA: McGraw Hill.

Tchobanoglous, G., Ruppe, L., Leverenz, H. & Darby, J. (2004) Decentralized wastewater management: challenges and opportunities for the twenty-first century. *Water Supply*. 4 (1), 95–102.

Tee, H.C., Lim, P.E., Seng, C.E. & Nawi, M.A.M. (2012) Newly developed baffled subsurface-flow constructed wetland for the enhancement of nitrogen removal. *Bioresource Technology*. 104, 235–242.

Tenaillon, O., Skurnik, D., Picard, B. & Denamur, E. (2010) The population genetics of commensal Escherichia coli. *Nature Reviews Microbiology*. 8 (3), 207–217.

Tihansky, D.P. (1974) Historical development of water pollution control cost functions. *Water Pollution Control Federation*. 46 (5), 813–833.

Troeger, C., Forouzanfar, M., Rao, P.C., Khalil, I., Brown, A., Reiner, R.C., Fullman, N., Thompson, R.L., Abajobir, A., Ahmed, M., Alemayohu, M.A., Alvis-Guzman, N., Amare, A.T., Antonio, C.A., Asayesh, H., Avokpaho, E., Awasthi, A., Bacha, U., Barac, A., et al. (2017) Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the Global Burden of Disease Study 2015. *The Lancet Infectious Diseases*. 17 (9), 909-948.

Uggetti, E., Hughes-riley, T., Morris, R.H., Newton, M.I., Trabi, C.L., Hawes, P., Puigagut, J. & García, J. (2016) Science of the Total Environment Intermittent aeration to improve wastewater treatment ef fi ciency in pilot-scale constructed wetland. *Science of the Total Environment, The*. 559, 212–217.

UK Technical Advisory Group on the Water Framework Directive (2009) *Recommendations on Surface Water Classification Schemes for the purposes of the Water Framework Directive*. London, UK.

United Nations (2018) *The Sustainable Development Goals Report 2018*. United Nations. New York, USA.

US EPA (2011) *Principles of Design and Operations of Wastewater Treatment Pond Systems for Plant Operators, Engineers, and Managers*. EPA/600/R-11/088. Cincinati, Ohio, USA.

Wang, S. (2014) Values of decentralized systems that avoid investments in idle capacity within the wastewater sector: A theoretical justification. *Journal of Environmental Management*. 136, 68–75.

Wanjugi, P., Sivaganesan, M., Korajkic, A., Kelty, C.A., McMinn, B., Ulrich, R., Harwood, V.J. & Shanks, O.C. (2016) Differential decomposition of bacterial and viral fecal indicators in common human pollution types. *Water Research*. 105591–601.

Ward, J.H. (1963) Hierarchical Grouping to Optimize an Objective Function. *Source Journal of the American Statistical Association*. 58 (301), 236–244.

Warish, A., Triplett, C., Gomi, R., Gyawali, P., Hodgers, L. & Toze, S. (2015) Assessment of Genetic Markers for Tracking the Sources of Human Wastewater Associated Escherichia coli in Environmental Waters. *Environmental Science and Technology*. 49 (15), 9341–9346.

Weber, B., Cornel, P. & Wagner, M. (2007) Semi-centralised supply and treatment systems for (fast growing) urban areas. *Water Science and Technology*. 55 (1–2), 349–356.

Wendland, C. & Albold, A. (2010) Sustainable and cost-effective wastewater systems for rural and peri-urban communities up to 10,000 PE. Guidance Paper. WECF. Utrecht, Netherlands.

Wickham, H. (2016) *ggplot2: Elegant Graphics for Data Analysis.* New York, NY: Springer-Verlag.

Wiggins, B.A. (1996) Discriminant analysis of antibiotic resistance patterns in fecal streptococci, a method to differentiate human and animal sources of fecal pollution in natural waters. *Applied and Environmental Microbiology*. 62 (11), 3997–4002.

Wilcox, J., Nasiri, F., Bell, S. & Rahaman, M.S. (2016) Urban water reuse: A triple bottom line assessment framework and review. *Sustainable Cities and Society*. 27, 448–456.

Wilson, F. & Lee, W.M. (1997) Rotating biological contactors for wastewater treatment in an equatorial climate. *Water Science and Technology*. 35 (8), 177–184.

Withers, P.J.A., Jarvie, H.P. & Stoate, C. (2011) Quantifying the impact of septic tank systems on eutrophication risk in rural headwaters. *Environment International*. 37 (3), 644–653.

Withers, P.J.A., May, L., Jarvie, H.P., Jordan, P., Doody, D., Foy, R.H., Bechmann, M., Cooksley, S., Dils, R. & Deal, N. (2012) Nutrient emissions to water from septic tank systems in rural catchments: Uncertainties and implications for policy. *Environmental Science and Policy*. 24, 71–82.

World Health Organisation (2017) *Guidelines for Drinking-water Quality. Fourth Edition Incorporating the First Addendum*. Geneva, Switzerland.

WPL (2019) *High performance aerated filter packaged wastewater treatment plants*. Online. Accessed, 01/12/2018.

Wu, S., Kuschk, P., Brix, H., Vymazal, J. & Dong, R. (2014) Development of constructed wetlands inperformance intensifications for wastewater treatment: A nitrogen and organic matter targeted review. *Water Research*. 57, 40–55.

Yamahara, K., Blythe A. Layton, Alyson E. Santoro, A. & Boehm*, A.B. (2007) *Beach Sands along the California Coast Are Diffuse Sources of Fecal Bacteria to Coastal Waters*. Environmental Science and Technology. 41 (13), 4515-4521.

Ye, F. & Li, Y. (2009) Enhancement of nitrogen removal in towery hybrid constructed wetland to treat domestic wastewater for small rural communities. *Ecological Engineering*. 35 (7), 1043–1050.

Young, P.C. & Beven, K.J. (1994) Data-based mechanistic modelling and the rainfall-flow non-linearity. *Environmetrics*. 5 (3), 335–363.

Zeileis, A. & Grothendieck, G. (2015) zoo: S3 Infrastructure for Regular and Irregular Time Series. *Journal of Statistical Software*. 14 (6), 1–27.

Zhang, Lie-yu, Zhang, Lan, Liu, Y., Shen, Y., Liu, H. & Xiong, Y. (2010) Effect of limited arti fi cial aeration on constructed wetland treatment of domestic wastewater. *DES*. 250 (3), 915–920.

Appendix A

List of Figures

List of Tables

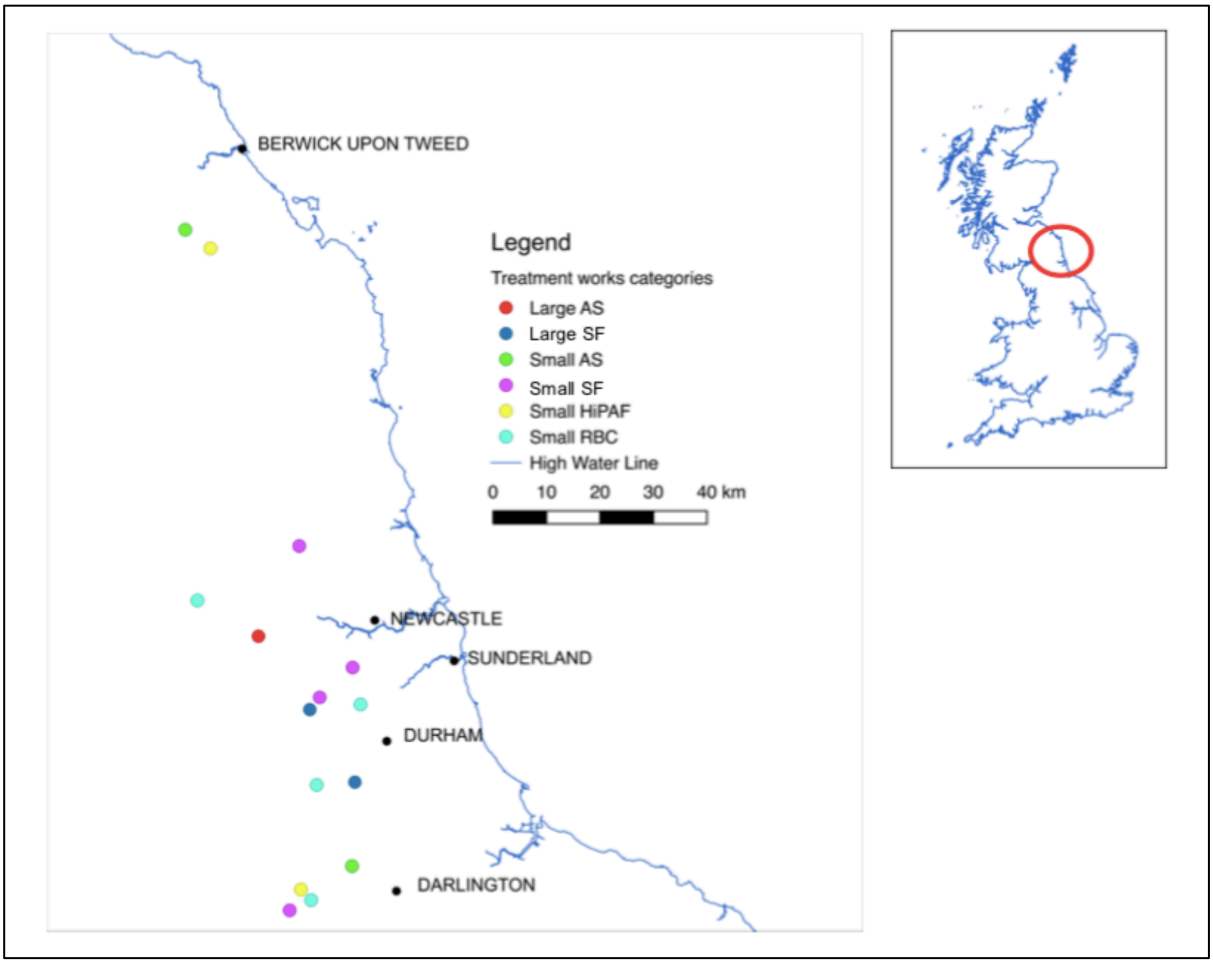


Figure A1 - Location of WWTPs sampled by treatment plant type. AS is activated sludge, SF is secondary filtration, HiPAF is high performance aerated filter and RBC is rotating biological contactor.

Site	Design category	PE	Flow monitoring
A	125-250_SF	161	No
B	50-125_SF	72	No
C	50-125_AS	89	No
D	50-125_SF	110	No
E	125-250_RBC	238	Yes
F	50-125_RBC	79	No
G	50-125_RBC	68	No
H	125-250_SF	128	No
I	125-250_HiPaf	199	Yes
J	50-125_AS	88	No
K	125-250_RBC	262	Yes
L	125-250_HiPaf	188	Yes
M	5000-10000_AS	5280	Yes
N	5000-10000_SF	7140	Yes
O	5000-10000_SF	9650	Yes

Table A1 – List of WWTPs included in field study and analysis presented in Chapters 3 and 4.

Appendix B

List of Tables

	Accuracy	Sensitivity	MSE
Random Forest	64.2%	0.92	0.36
Gradient Boosting Machine	47.6%	0.42	0.52
Generalised Linear Model	52.4%	0.67	0.48

Table B1 - Performance criteria of three models tested to predict the reliability of small WWTPs. MSE is the mean standard error.

		Reference	
		Actual > Design	**Actual < Design**
Prediction	**Actual > Design**	41.67 %	44.44 %
	Actual < Design	58.33 %	55.56 %

Table B2 - Confusion matrix for gradient boosting machine when predicting the reliability of small WWTPs

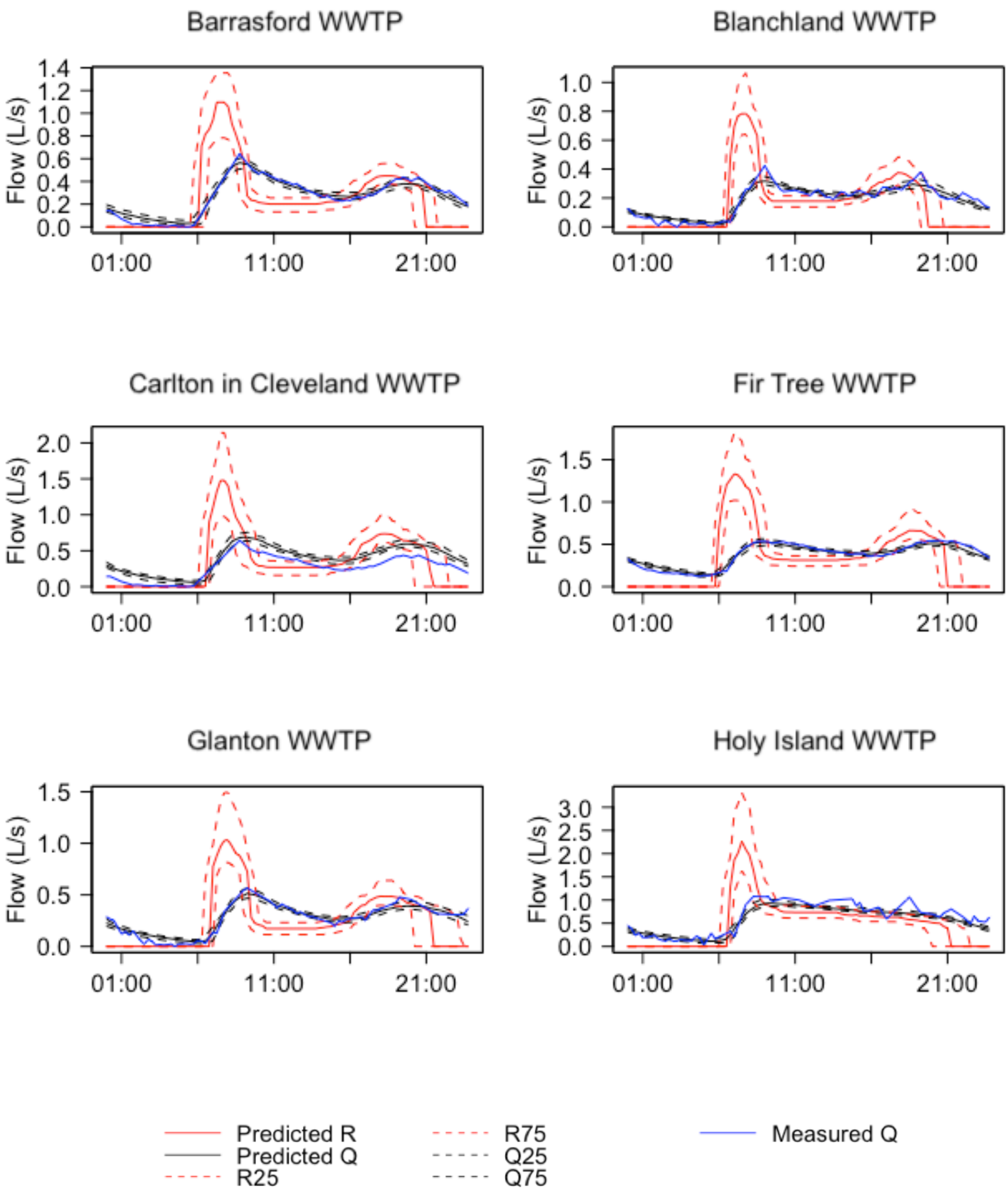
		Reference	
		Actual > Design	**Actual < Design**
Prediction	**Actual > Design**	66.67 %	66.67 %
	Actual < Design	33.33 %	33.33 %

Table B3 - Confusion matrix for gradient boosting machine when predicting the reliability of small WWTPs

Appendix C

List of Figures

Figure C1 – Predicted diurnal flow profiles for each WWTP



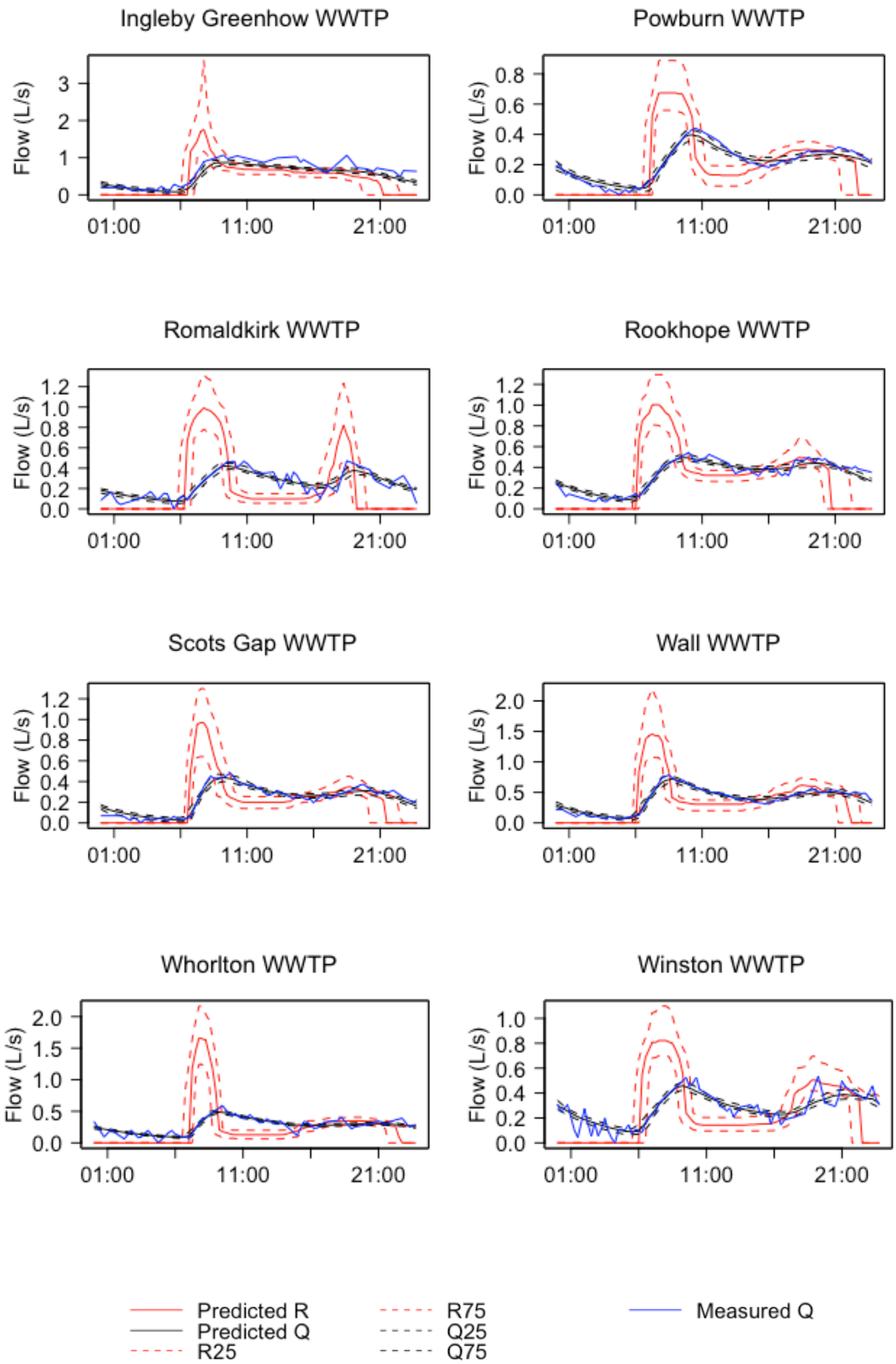


Figure C1 – Predicted dry weather flow profiles for each WWTP shown alongside measured mean and predicted human contributions. Q is flow, R is human generated flow. Upper and lower quartiles denoted as 75 and 25, respectively.

THE END