PATHOGEN INFECTION RISK TO RECREATIONAL WATER USERS,
ASSOCIATED WITH SURFACE WATERS IMPACTED BY *DEFACTO AND*
INDIRECT POTABLE REUSE ACTIVITIES

Sarah Purnell\textsuperscript{a*}, Andrew Halliday\textsuperscript{b}, Freya Newman\textsuperscript{a}, Christine Sinclair\textsuperscript{a}, and James Ebdon\textsuperscript{a}

\textsuperscript{a}Environment and Public Health Research and Enterprise Group, Centre for Aquatic Environments, School of Environment and Technology, University of Brighton, Cockcroft Building, Lewes Road, Brighton, BN2 4GJ, United Kingdom

\textsuperscript{b}South East Water Ltd. Rocfort Road, Snodland, Kent, ME6 5AH

*Corresponding author phone: +44 1273 641344; e-mail: S.E.Purnell@Brighton.ac.uk;
address: University of Brighton, Cockcroft Building, Lewes Road, Brighton, BN2 4GJ,
United Kingdom

© 2020. This manuscript version is made available under the CC-BY-NC-ND 4.0 license
http://creativecommons.org/licenses/by-nc-nd/4.0/

Competing Financial Interests

The authors declare they have no actual or potential competing financial interests.

Abstract

Water deficit, exacerbated by global population increases and climate change, necessitates the investigation of alternative non-traditional water sources to augment existing supplies. Indirect potable reuse (IPR) represents a promising alternative water source in water-stressed
regions. Of high concern is the presence of pathogenic microorganisms in wastewater, such as enteric viruses, protozoa and bacteria. Therefore, a greater understanding of the potential impact to human health is required. The aim of this research was to use a quantitative microbial risk assessment (QMRA) approach to calculate the probability of potential pathogen infection to the public in surface waters used for a range of recreational activities under scenarios: 1) existing de facto wastewater reuse conditions; 2) after augmentation with conventionally treated wastewater; and 3) after augmentation with reclaimed wastewater from proposed IPR schemes. Forty-four 31 L samples were collected from river sites and a coastal wastewater treatment works from July 2016–May 2017. Concentrations of faecal indicator organisms (enterococci, faecal coliforms, somatic coliphages and Bacteroides phages) determined using culture-based approaches and selected pathogens (adenovirus, Salmonella and Cryptosporidium) determined using molecular approaches (qPCR) were used to inform QMRA. The mean probability of infection from adenovirus under de facto conditions was high (>0.90) for all recreational activities, per single event. The risk of adenovirus and Cryptosporidium infection increased under augmentation scenario (2) (mean probability 0.95-1.00 and 0.01-0.06 per single event, respectively). Adenovirus and Cryptosporidium infection risk decreased under reclaimed water augmentation scenario (3) (mean probability <0.79, excluding swimming, which remained 1.00 and <0.01 per single event, respectively). Pathogen reduction after reclaimed water augmentation in surface waters impacted by de facto reuse, provides important evidence for alternative water supply option selection. As such, this evidence may inform water managers and the public of the potential benefits of IPR and improve acceptance of such practices in the future.

Key words
water reuse, adenovirus, Cryptosporidium, Salmonella, human health, recreation,
augmentation

**Introduction**

Treated municipal wastewater is being increasingly recognised as a valuable and sustainable resource that can supplement more conventional water supplies. This is particularly important as population growth and climate change are predicted to place additional strain on finite freshwater supplies. In principle, wastewater can be collected and treated, removing human pathogens and other contaminants to produce a reusable product (Wintgens et al., 2008; Asano and Cotruvo, 2004; Salgot et al., 2006; Purnell et al., 2016). In many parts of the world wastewater reuse systems (both indirect and direct) have been successfully implemented, provided that the potential risks to human health are fully understood and adequately controlled. Of particular concern are pathogenic microorganisms such as enteric viruses commonly found in municipal wastewaters (Dias et al., 2018). Water reuse is actively encouraged in the EU Urban Wastewater Treatment Directive (91/271/EEC) and the EU Water Framework Directive (2000/60/EC). As a result, multiple EU Member States have developed varying guidelines for wastewater reuse. International reuse guidelines have also been developed by many other countries (US EPA, 2012 and the EPHC, 2008).

According to the US EPA (2012), *de facto* wastewater reuse is defined as the reuse of treated wastewater that is not officially recognised. *De facto* wastewater reuse is widespread and drinking water supply intakes are frequently located downstream of wastewater treatment discharge points. Indirect potable reuse (IPR) on the other hand is the process of augmentation of ground or surface water drinking sources with reclaimed wastewater, where an environmental buffer precedes a drinking water supply intake. The environmental buffer
can provide storage, transport and may act as an additional barrier for the protection of public health (U.S. EPA, 2012).

Significant global population increases and projections for water availability indicate that water deficits will become an increasing problem worldwide and wastewater discharge volumes will increase. For example, projections for water availability for the UK indicate that even under lower bound scenarios (a low population and medium climate change projection) there will be significant water deficits in the south-east of England and elsewhere by 2050 (HR Wallingford, 2015). Projected water deficits have meant that water managers must investigate alternative non-traditional sources, with which to augment water supplies. IPR represents a promising alternative water source, providing a sustainable supply and a potential reduction of pollutant release into the environment. Whilst it is recognised that advanced treatment technology makes it possible to treat wastewater (even for direct potable reuse (DPR)) to the standard of intended use (US EPA, 2012), it is also important to limit where possible the associated costs, energy consumption and carbon output, as this has implications for the viability and sustainability of a proposed scheme.

River catchments are typically used throughout the year by a range of stakeholders and end-users for many different (sometimes conflicting) uses including recreational activities, such as swimming and kayaking. Experience has shown that public opposition can be a significant barrier to the successful implementation of wastewater reuse schemes (Hurlimann and Dolnicar, 2009; Fielding, Dolnicar and Schultz, 2019) and customer satisfaction is an important consideration for water companies. Therefore, in order to make informed decisions considering the introduction of wastewater reuse schemes and to alleviate public health concerns and improve confidence, a greater understanding of the potential impact to human
health is required. Whilst research has focused on the risk to drinking water consumers, less
is understood about how existing *de facto* reuse and proposed IPR schemes impact the health
and wellbeing of recreational users of source waters. Suitable methods for assessing
recreational health risk include the detection of faecal indicator bacteria (FIB) (coliform
bacteria, and intestinal enterococci), bacteriophages (coliphages or *Bacteroides* phages) or the
direct detection of pathogens of human health significance. FIB and bacteriophage indicator
have simpler detection methods and are less expensive to monitor than the pathogens
themselves (Field et al., 2007), and are capable of providing an indication of faecal
contamination and the likely human health risk arising from ingestion of water.
Bacteriophages have been shown to better correlate with the presence of enteric viruses than
FIB, and they may also offer important information on likely sources of faecal contamination
(Purnell et al., 2011, 2018; Ebdon et al., 2012). Concentrations of FIB, bacteriophages and
pathogens have been used to inform quantitative microbial risk assessment (QMRA) of
various wastewater reuse scenarios (Liu and Persson, 2014; King et al., 2017). QMRA is an
approach that uses the principles of risk assessment to estimate the consequences from
exposure to infectious microorganisms under different scenarios. QMRA of microorganisms
can help elucidate the exposure pathways and the risks associated with different water
sources, applications, and uses, whilst also providing a detailed breakdown of each
contributing step to reduce overall risk (Haas, 1999).

IPR schemes have significant advantages, including a reduction in pollutants discharged into
the environment at wastewater treatment sites and the introduction of significant additional
water sources. Inland surface water abstraction sites are already frequently impacted by *de
facto* wastewater reuse, being located downstream of numerous wastewater treatment
discharges of varying magnitude. Inland surface waters are also increasingly popular with the
public for recreational pursuits (including swimming and boating). The combination of different uses within inland surface waters makes the investigation of human health risks to recreational users from existing de facto reuse activities critical. In addition, it is important to predict the impact of the introduction of IPR schemes on the existing human health risk, as water resource managers search for alternative water sources. Therefore, the aim of this research was to quantify reference bacterial, viral and protozoan pathogens of human health concern and to use a QMRA approach to calculate the probability of potential pathogen infection for recreational users under: 1) de facto reuse conditions; 2) after additional augmentation with conventionally treated wastewater; 3) after additional augmentation with reclaimed wastewater subjected to advanced treatment through planned IPR schemes. There is currently very limited evidence in the literature, of the impact of de facto and IPR augmentation on surface water quality and microbial risk to the public through recreation. De facto reuse is a common practise globally and IPR represents an important alternative water source for many countries. Therefore, the findings of this research contribute important empirical evidence of the risk to recreational users in scenarios relevant worldwide. As such, empirical data from this research will help water resource managers to make informed decisions on whether to include and select IPR schemes as options for the provision of alternative water sources in water management plans. The evidence presented could also be used to inform the public of the benefits of such reuse schemes, increasing public acceptance through better understanding of the processes and risks involved.

Methods

Monitoring programme.

Thirty-three surface water samples (31 L each time) were collected at a drinking water abstraction site (site X) and at two proposed IPR augmentation sites located 1.5 km (site Y)
and 3 km upstream (site Z) in a catchment in the South East of England. Samples were collected in sterile 10 litre polyethylene sampling containers and 1 litre sampling bottles over an eleven-month period (July 2016–May 2017) (Figure 1). Eleven wastewater effluent samples (31 L each time) were also collected from a coastal wastewater treatment works (site W) (pop. equiv. 293,165) over the same period (July 2016–May 2017). The treatment works has a pre-treatment facility that removes fat, oil, grease and grit. The wastewater undergoes primary treatment through Multifloë lamella clarifiers and then secondary treatment through Biostyrë biological aerated flooded filters. All samples (n=44) were tested for the presence of thermotolerant coliforms, intestinal enterococci, somatic coliphages (bacteriophages that infect *Escherichia coli* through the cell wall), and human-specific bacteriophage (capable of infecting *Bacteroides* host strain GB124) using culture-based approaches and pathogenic organisms (adenovirus, *Salmonella* and *Cryptosporidium*) using qPCR. Catchment and specific site names are not given due to confidentiality agreements.

**Indirect potable reuse scheme proposal.**

A proportion of the effluent from a coastal wastewater treatment works is proposed to be used as part of an IPR scheme and would be treated at the existing treatment site, following the construction and commissioning of a new water reclamation facility. Reclaimed wastewater will then be transferred underground to one of two proposed augmentation sites (site Y or Z). The proposed water reclamation facility would consist of coagulation, clarification, filtration, ozone, biological activated carbon, granular activated carbon and ultraviolet + ultrafiltration and reverse osmosis side-stream. This is intended to provide multiple barrier treatment and 12, 10, 10-log removal for enteric virus, *Cryptosporidium*, and *Giardia*, respectively (log reduction values implemented in California for groundwater injection with reused wastewater) (WateReuse, 2014).
**Quantification of faecal indicator organisms.**

Thermotolerant coliforms (TTC) and intestinal enterococci (IE) were enumerated in duplicate by membrane filtration on mFC agar (Difco) and Slanetz and Bartley agar (Oxoid), respectively, in accordance with standard methods (Anon, 2000). All results were expressed as colony-forming units per ml (CFU/ml). Somatic coliphages (SC) and human-specific bacteriophage (capable of infecting Bacteroides host strain GB124) were quantified by enumerating plaque-forming units (PFU/ml), in duplicate on Modified Scholtens agar (MSA) and Bacteroides Phage Recovery Media agar (BPRMA), respectively, according to standardised double-agar-layer methods (Anon, 2001a,b). Host strain WG5 was used for SC enumeration and strain GB124 was used for the detection of bacteriophages active against Bacteroides fragilis.

**Quantification of pathogens (bacteria, protozoa and viruses).**

Salmonella (bacterial pathogen), Cryptosporidium (protozoal pathogen), and human adenovirus (viral pathogen) were enumerated using quantitative real-time polymerase chain reaction (qPCR) assays as outlined below.

**Concentration for detection of pathogenic viruses.**

Ten litre samples were concentrated for human adenovirus (AdV type F and G) using a skimmed milk flocculation procedure, as described in Purnell et al. (2016) and Calgua et al. (2013). Before sample concentration, conductivity was measured and altered to achieve levels greater than 1.5 µS/cm using sterile artificial sea salts (Sigma-Aldrich, UK). The pH of the sample was also reduced to 3.5 by adding HCL 1 N. Once the samples were prepared, a 1% (w/v) pre-flocculated skimmed milk solution (PSM) was created by dissolving 10 g
skimmed milk powder into 1 L of artificial sea salt solution at a pH of 3.5. The PSM was added to each sample to achieve a final concentration of 0.01%. Samples were stirred for 8 h at room temperature using a magnetic stirrer, followed by an additional 8-10 h of settling to allow flocs to sediment by gravity. The supernatant was removed using a syphon, avoiding disturbance of the settled flocs, leaving a final volume of approximately 500 ml. This was then centrifuged at 7000-8000 x g for 30 min at 4°C. The supernatant was carefully removed and the pellet re-suspended in 10 ml of phosphate buffer (1:2, v/v of Na2HPO4 0.2 M and NaH2PO4 0.2 M) at pH 7.5, at a ratio of 1 mL of phosphate buffer per 1 L of concentrated sample. The viral concentrate was stored at -80 °C.

**Concentration for detection of pathogenic bacteria and protozoa.**

To concentrate pathogenic bacteria (*Salmonella*) and protozoa (*Cryptosporidium*), 10 L samples were filtered through 0.45µm pore size nitrate-cellulose filter membranes (Sartorious) and cellulose-acetate membranes with a pore size of 3 µm (Advantec), respectively as described by Ahmed et al. (2008). The filters were immediately placed into 15 ml screw cap centrifuge tubes containing 10 ml Phosphate Buffered Saline (PBS) and vortexed vigorously for 5 min to detach the organisms from the membranes. Samples were then centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was discarded and the pellet re-suspended in 2 ml of sterile distilled water.

**Nucleic acid extraction.**

Nucleic acid extractions were performed using the genesig® Easy DNA/RNA Extraction Kits (Primer Design, UK) according to the manufacturer’s instructions. DNA was extracted from 200µl of concentrated sample. Nucleic acids were stored at -80 °C until further analysis (within 4 months of freezing).
**Quantitative real-time PCR assays.**

All qPCR assays were performed using a Qiagen Rotar-gene Q. ‘Positive’, ‘negative’ and ‘internal extraction’ controls were used in every assay run. Dilutions were used to limit inhibition in samples. Genesig® kits (Primer Design, UK) were used for the detection of adenovirus (AdV) type F and G, and pathogenic strains of *Salmonella* and *Cryptosporidium*. Amplification conditions for all pathogens consisted of enzyme activation for 2 mins at 95 ºC, 50 cycles of denaturation for 10 s at 95 ºC and data collection for 60 s at 60 ºC.

**Quantitative Microbial Risk Assessment (QMRA).**

This QMRA included four principal steps: (1) hazard identification, (2) exposure assessment, (3) effect assessment (dose-response) and (4) risk characterisation. Potential pathogen infection risk for the public using the river sites for recreational activities was calculated under existing *de facto* wastewater reuse conditions (scenario 1). These results were then compared to risk calculations for two potential future scenarios: 2) river water augmentation with conventionally treated wastewater from the coastal wastewater treatment works (to simulate future increases in *de facto* wastewater discharge associated with increasing population) and 3) river water augmentation with reclaimed water after further treatment through the proposed water reclamation facility. Scenarios 2 and 3 were calculated for a range of augmentation scenarios (assuming augmentation proportions of 0, 10, 20, 30, 40 and 50% of the total river flow). In summer months during 2016 and 2017 mean flows at the river sites were 37% below mean winter flow rates. The minimum flow rate recorded was 86% less than mean winter flow rates. Therefore, proposed augmentation scenarios fall within a realistic range for this catchment.
Hazard identification.

Table 1 details the selected pathogens (hazards) for this QMRA (Adenovirus, Cryptosporidium and Salmonella).

Exposure assessment.

Exposure assessment is a step that calculates the dose of a pathogen that an individual is likely to ingest, inhale or comes into contact with during normal water usage. This number feeds into the subsequent ‘dose response’ models that predict the overall probability of infection. Primary exposure was calculated in this study. Secondary exposure via infected individuals, or fomites was not considered in these calculations. The calculations in this research also assume that microbial inactivation does not significantly attenuate pathogen concentrations from the point of augmentation (1.5km or 3km upstream) to the drinking water abstraction site (site X). The method used for estimating exposure dose is presented below.

\[ D = C \cdot V \cdot T \]

Where \( D \) is the exposure dose, \( C \) is the concentration of microorganism, \( V \) is the amount of the contaminant to which a person is exposed and \( T \) is the exposure duration.

Pathogen concentrations were determined using Monte Carlo simulations of triangular probability distributions that could not go lower than the minimum observed concentration, or exceed the maximum concentrations observed. Because modal data did not exist, triangular distributions were assumed to be symmetrical and the mean was used as the most likely value. As a result, all values produced by the simulations were within the range of
observed concentrations from the monitoring data. The calculated pathogen concentrations were used for exposure calculations, with assumed augmentation with either conventionally treated wastewater or reclaimed water in proportions of 10%, 20%, 30%, 40% and 50% of the total water volume in the river. Estimated volumes of water ingested during relevant recreational activities were taken from the extant scientific literature. According to the World Health Organisation (WHO), the estimated volume of water consumed whilst swimming in a river is 20-50ml/ h (2003). Rowing, canoeing, and kayaking consumption rates have been estimated at 3.5, 3.9 and 3.8 ml/ h, respectively and ingestion during fishing has been estimated at 3.6 ml/ h (Dorevitch et al., 2011; Schets et al., 2011). It was assumed that exposure was a single event.

**Effect assessment.**

The effect assessment is the stage where risk of infection is calculated according to calculated pathogen doses (*exposure assessment*). Dose response models are mathematical functions that describe the dose relationship for particular pathogens, transmission routes and hosts. Table 2 presents the selected parameters for dose response models for adenovirus, *Salmonella* and *Cryptosporidium* pathogens. The best-fit models shared by the Center for Advancing Microbial Risk Assessment (CAMRA) were used to calculate dose-response for all pathogens. The exponential and Beta-Poisson dose-response models selected are shown below (CAMRA, 2011).

**Exponential model:**

\[ P(\text{response}) = 1 - \exp(-k \times \text{dose}) \]

**Beta – Poisson model:**

\[ 1 - \left[ 1 + \text{dose} \left( \frac{1}{2\pi-1} \right) \right]^{-\alpha} \]
Where \( \text{exp} = \) exponential, \( \text{dose} = \) calculated exposure dose, \( N_{50} \) represents the dose at which 50% of the population is expected to be affected, Values for \( k \) represent the survival probabilities.

**Risk characterisation.**

Risk characterisation combines the information from exposure assessment and effect assessment to determine the probability of infection per person per year. This was stochastically estimated using the software @Risk version 7.5.1 (Palisade Corporation). Risk of infection was calculated for a single event (an activity undertaken once) and annually assuming that an individual would partake in the select activity once per month.

**Statistical analysis.**

All data distributions for parameters were analysed for normality. Non-parametric statistical tests were used because the data were not normally distributed. To determine if there were statistically significant differences between the water quality of the river sites (Sites X, Y and Z), Kruskal-Wallis statistical tests were used. For comparison between river site data and treated wastewater data the Mann-Whitney test was used. Correlation analysis was performed using the Spearman’s Rank correlation coefficient. The statistical tests described were conducted with the statistical software Minitab version 19 with a significance level set at 5%. The results of statistical tests are presented in brackets with the P value result to support the interpretation within the text.

**Results**

**Monitoring of faecal indicator organisms.**
Figure 2 presents the concentrations of faecal indicator organisms at river sites and in treated wastewater (intestinal enterococci, faecal coliforms, somatic coliphages, and human-specific bacteriophage capable of infecting *Bacteroides* host strain GB124) between 25th July 2016 and the 15th May 2017. Concentrations of faecal indicator organisms were compared across the river sites (the drinking water abstraction site (site X) and proposed reclaimed wastewater augmentation sites (site Y – 1.5 km upstream and site Z – 3 km upstream of the drinking water abstraction site). Statistically there was no significant difference between the concentrations of intestinal enterococci, somatic coliphages and phages infecting human-specific *Bacteroides* strain GB124 between the river sites (Kruskal-Wallis: P-value = 0.152, 0.907 and 0.577, respectively). Whilst there was a significant difference between concentrations of thermotolerant coliforms across the river sites (Kruskal-Wallis; P-value =0.034), the observed difference in concentrations of 0.58 log CFU/ml was relatively small. The faecal indicator organism concentrations in river sites were then compared to concentrations in treated wastewater. Data from all river water samples were grouped together, since differences between the sites were insignificant. Concentrations of intestinal enterococci, thermotolerant coliforms, somatic coliphages and phages infecting human-specific GB124 were significantly higher in treated wastewater (Mann-Whitney; P-value = 0.00, 0.00, 0.02 and 0.00, respectively). Median concentrations of intestinal enterococci were between 1.74 and 1.92 log CFU/ml greater and thermotolerant coliforms 1.7 and 2.28 log CFU/ml greater in treated wastewater, compared with river water samples. Median concentrations of somatic coliphages were also between 1.30 and 1.44 log PFU/ml greater in treated wastewater. Concentrations of phages infecting human-specific GB124 were low in river water samples, with a median of <0.01 log PFU/ml, whereas the median concentration in treated wastewater was 0.78 log PFU/ml.
Figure 3 presents the concentrations of pathogens (adenovirus, *Salmonella* and *Cryptosporidium*) at river sites and in treated wastewater. There was no statistically significant difference in concentrations of adenovirus or *Salmonella* between the river sites (Kruskal-Wallis; P-value = 0.39 and 0.970, respectively). Concentrations of *Salmonella* were not significantly different between river sites and treated wastewater (Mann-Whitney; P-value = 0.75), with relatively low concentrations in both river water samples and treated wastewater (medians both <0.01 log copies/L). According to statistical analysis, concentrations of adenovirus were significantly higher in treated wastewater (Mann-Whitney; P-value = 0.04), with concentrations between 0.04 and 0.73 log copies/L higher. However, it is noteworthy that median concentrations of adenovirus at one of the proposed augmentation sites (Site Z - 3km upstream of the drinking water abstraction site) were only 0.04 log copies/L lower than in the treated wastewater. *Cryptosporidium* was only detected in a single sample from treated wastewater (n=11) at 2.10 log copies/L (October 17th, 2016) and not in river water samples tested (n= 33).

**Correlation between indicator organisms and pathogens.**

The Spearman’s Rank correlation coefficient was used to determine the significance and strength of correlation between the indicator and pathogenic organisms monitored within this study (Table 3). Results demonstrated that only somatic coliphages correlated with adenoviruses. A significant moderate-strength positive ($r^2= 0.426$) correlation was observed between these organisms (p-value of 0.004). No other faecal indicator organism correlated with *Salmonella* or adenovirus and somatic coliphages did not correlate with *Salmonella*. *Cryptosporidium* was not included in correlation analysis, because there was only a single detection in treated wastewater from the coastal wastewater treatment works.
Quantitative microbial risk assessment

Variation in the data from the river sites (drinking water abstraction site (site X) and proposed wastewater augmentation sites (sites Y and Z)) was statistically insignificant, therefore all river site water quality data for QMRA was combined (sample n=33), increasing the robustness of the dataset. The mean probabilities of potential infection from adenovirus, Cryptosporidium and Salmonella were calculated for a range of augmentation scenarios (assuming discharge proportions of 0, 10, 20, 30, 40 and 50% of the total river flow), and the different recreational activities, through which the public may come into contact with surface water at the river sites (X, Y and Z). Stochastic simulations were performed with 10,000 iterations to determine the mean probability of infection.

Scenario 1: existing infection risk to recreational users.

A QMRA of potential pathogen infection risk to recreational users was undertaken using water quality monitoring data from the river sites (X, Y and Z) (scenario 1). The results are shown (in Figures 4, 5, 6, and 7) as infection risk for recreational activities under the scenario of zero additional wastewater/reclaimed water discharge. These results are compared in these figures to the risk under each of the two future scenarios (scenario 2 and 3). The probability of infection from adenovirus under de facto conditions was high (>0.90) for all recreational activities per single event. Swimming represented the greatest risk of adenovirus infection with a mean probability of 1.00 per single event. Infection risk for Cryptosporidium and Salmonella was found to be negligible (<0.01) under existing de facto reuse (scenario 1).

Scenario 2: augmentation with conventionally treated wastewater.
A QMRA of potential pathogen infection risk after additional conventionally treated wastewater augmentation (10-50% proportion of total mean daily flow) was undertaken using treated wastewater quality data from the proposed IPR wastewater source (the coastal wastewater treatment works). Figures 4, 5 and 6 present the results of the QMRA for recreational activities for adenovirus and Cryptosporidium. The QMRA for Salmonella is not presented, as low concentrations of Salmonella and a higher dose required for infection meant that there was a negligible risk to recreational users in all scenarios (<0.01).

The mean probability of infection with adenovirus from all recreational activities (swimming, canoeing, kayaking, fishing and rowing) was high under all conventionally treated wastewater augmentation percentage scenarios (>0.95) per single event (Figure 4). The mean probability of infection for each swimming event was 1.00 regardless of the percentage of treated wastewater used in augmentation and remained 1.00 even when additional augmentation was removed from the scenario (i.e. just considering de facto reuse). For canoeing, kayaking, fishing and rowing the mean probability of infection remained high when treated wastewater augmentation was removed from the scenario (0.91, 0.91, 0.90 and 0.90, respectively). The mean annual probability of infection for adenovirus remained 1.00 for all recreational activities (assuming the activity was conducted once a month). The risk of infection from Cryptosporidium was considerably lower than that of adenovirus. Cryptosporidium was only detected once in treated wastewater, so the risk of infection when there was no additional treated wastewater contribution was <0.01 for all recreational activities. The risk increased as the proportion of wastewater to the river water increased (from 10% to 50%). Swimming had the highest risk of infection (per single event), with a mean probability of 0.01 at 10%/90% augmented conventionally treated wastewater to river water to 0.06 at 50%/50% augmented conventionally treated wastewater to river water.
(Figure 5). For all other recreational activities the mean probability of infection was calculated at <0.01, with the exception of canoeing (0.01) at 50%/50% augmented conventionally treated wastewater to river water. For swimming the risk increased considerably when calculated annually for those swimming at least once a month from a mean probability of 0.14 at 10% to 0.49 when 50% of the flow was composed of augmented conventionally treated wastewater (Figure 6). Whilst the risk increased for all other recreational activities (canoeing, kayaking, fishing and rowing) when considering the annual risk (rather than per single event), the mean probability never exceeded 0.06.

**Scenario 3: augmentation with reclaimed water.**

A QMRA of potential pathogen infection risk after additional reclaimed water augmentation (10-50% proportion of total mean daily flow) was undertaken using treated wastewater quality data from the proposed IPR wastewater source (the coastal wastewater treatment works) with calculated pathogen reduction after multiple barrier treatment designed to result in a 12, 10, 10-log removal for enteric virus, *Cryptosporidium*, and *Giardia*, respectively. QMRA data for *Cryptosporidium* and *Salmonella* are not presented because the calculated risks were negligible to recreational users in all scenarios (<0.01 mean probability). Figure 7 presents the results of the QMRA for recreational activities at river sites for adenovirus.

In this scenario the reclaimed water quality was calculated to be of higher microbiological water quality than existing river water quality. This resulted in dilution of the pathogen numbers in the river water. Regardless, the mean probability for infection with adenovirus when swimming remained 1.00 per single event (and annually) regardless of the proportion of reclaimed water to river water. However, adenovirus infection risk did decrease for all other recreational activities. Adenovirus mean probability of infection per single event for
canoeing, rowing, fishing and kayaking decreased from 0.91, 0.90, 0.90 and 0.91 to 0.78, 0.75, 0.76 and 0.77, respectively. The annual risk of infection with adenovirus remained constant, with a mean probability of 1.00 for all recreational activities, assuming the activity was conducted once a month.

Discussion

Globally, river catchments have multiple (often conflicting) uses and are commonly used for a variety of recreational activities (some involving direct contact/immersion with the water). For example, in the UK, the popularity of activities such as wild swimming continues to grow, whilst most inland river sites used for recreation are not protected by bathing water designation (under the Revised Bathing Water Directive (2006)). De facto wastewater reuse is commonplace throughout much of the world and future predicted water deficits encourage the drinking water providers to diversify water sources and to investigate non-traditional supply options. IPR schemes would enable water resource managers to augment water during low flow periods. These low flow periods are most likely to occur in summer months, aligning with periods of the year when recreational users (particularly swimmers) are most likely to use river sites, compounding any potential human health risk. This research provides evidence of the existing potential pathogen infection risk from recreational use of river sites impacted by existing de facto wastewater reuse activities. Predictions of the pathogen risk that additional conventionally treated wastewater augmentation or reclaimed water augmentation from a proposed IPR scheme poses are also presented. One limitation that should be considered is that there is no consideration of the implications of complete or partial treatment failures in the conventional wastewater treatment works in this research, nor of the potential impact of thermal or chemical contaminants (e.g. pharmaceutical or personal care products) on human or environmental health within such river systems.
The mean FIB concentrations detected in this study were compared to the Revised Bathing Water Directive (2006) standards and indicate that the river sites would currently fall into the ‘excellent’ bathing water category for inland and transitional waters. Maximum values suggested that thermotolerant coliform concentrations fall into a ‘good’ quality instead of ‘excellent’ on only two separate occasions. In contrast, FIB were observed at ten times these concentrations in treated wastewater from the coastal wastewater treatment works. Greater concentrations in treated wastewater were also evident for the bacteriophage-based indicators (somatic coliphages and bacteriophages infecting human-specific Bacteroides host strain GB124). Human-specific Bacteriodes (GB124) indicated that a greater contribution of human faecal material was present in treated wastewater as opposed to the river sites (which contain non-human contamination inputs) as would be expected. Human sources of faecal contamination are assumed to pose a greater risk to human health than non-human sources because they contain pathogenic organisms that have evolved to specifically infect humans (Soller et al., 2010). However, evidence from the QMRA in this study, indicated that river water designated as ‘good’ or ‘excellent’ under the Revised Bathing Water Directive (2006) did not correspond to a low risk of infection from adenovirus. Research has shown that bacterial indicators can predict the probable presence of pathogens, but that variation in input, dilution and die-off result in conditions that impact correlation (Payment and Locas, 2010).

These results suggest that bacterial standards set out in the EU Bathing Water Directive (2006) may be less suitable for determining the risk of infection from viral pathogens, such as adenovirus. FIB did not correlate significantly with the presence of adenovirus in this study, supporting the QMRA evidence. Interestingly, no significant correlation was evident between FIB and Salmonella. It should be noted that a reason for reduced correlation could be the different use of culture-based and molecular methods to detect faecal indicator organisms and
pathogenic organisms, respectively. However, these findings do imply that alternative indicators such as bacteriophages should be considered for determining the risk of viral pathogen infection. This is supported by correlation analysis that found a significant positive correlation between somatic coliphages and adenovirus in this study (p-value =0.004; r^2= 0.426). Increasing recognition of the limitations of FIB to predict viral pathogens has led to the growing use of coliphages (bacteriophages that infect *Escherichia coli*) in water regulation. As an example, the United States Environmental Protection Agency (USEPA) has reviewed and is exploring the introduction of coliphages as possible indicators of faecal contamination for surface water quality (USEPA, 2015).

For *Salmonella* the risk of infection under all scenarios was shown to be negligible. *Salmonella* is a bacterial pathogen and is therefore likely to inactivate more readily through wastewater treatment and in the environment, which could explain the lower concentrations detected in this research in comparison to adenovirus (Moce-Llivina et al., 2005).

*Cryptosporidium* was only detected in treated wastewater. Wastewater often contains human and non-human animal faecal sources from livestock and wildlife (including rodents in the wastewater network and birds at wastewater treatment sites). So, whilst *Cryptosporidium* is a zoonotic pathogen, its presence in treated wastewater is not unusual. Zahedi et al (2018) summarises the results of 27 studies (including the results of their study) that assess the prevalence of *Cryptosporidium* in wastewater. These studies conducted across Africa, Asia, Australia, Europe, North America and South America observed *Cryptosporidium* prevalence in wastewater ranging from 6.4-100%. QMRA scenario 2 (after augmentation with conventionally treated wastewater) indicated an increased risk of infection with *Cryptosporidium*. Whilst the risk remains moderate, the introduction of any treated wastewater has the potential to introduce a risk that was not previously present according to
our monitoring results (in 10L samples). Low concentrations of *Cryptosporidium* are still a concern as infectivity studies and dose-response modelling have shown that single oocyst infection probabilities can be up to 72% (Messner and Berger, 2016). *Cryptosporidium* is resistant to chlorination commonplace in conventional treatment works and requires an alternative treatment for removal, such as filtration and secondary disinfection with UV light or ozone (Betancourt and Rose, 2004; Hijnen et al., 2006; Zhang and Farahbakhsh, 2007; Chaudhry et al., 2015; Purnell et al., 2016; Chalmers et al., 2019). In contrast, the model suggests that the risk of infection from adenovirus under existing *de facto* reuse conditions was much higher ($\geq 0.90$) and did not increase substantially with augmentation with additional conventionally treated wastewater. Viral and protozoan micro-organisms are known to be more resistant to common wastewater treatment processes and the data presented here supports this understanding. The findings presented here indicate that advanced treatment to remove these organisms would be required to avoid further deterioration of water quality at river sites (X, Y and Z). The QMRA model results suggest that the treatment trains proposed for the water reclamation facility, would produce a far superior microbiological water quality than is currently observed at the river sites featured in this study. Discharge of this quality of water, would act to dilute existing pathogens in the river and reduce the risk of infection to recreational users. The planned water reclamation facility also adheres to current international standards for wastewater reuse for the purposes of augmentation, that require the absence of coliphages (viral indicators) and *Cryptosporidium* (U.S. EPA, 2012; EPHC, 2008; UKWIR, 2005). Research from Chaudhry et al. (2017) has shown similar outcomes when modelling the infection risks associated with the blending of *de facto* reuse waters with direct potable reuse (DPR) waters (finished product). Their results indicated that risk was affected mostly by contamination levels in surface water sources and not by the DPR treatment trains. Although not directly considered
here, an improvement in water quality could also have positive implications for water
treatment costs at the drinking water abstraction site (site X).

This study demonstrates the potential for pathogen reduction through augmentation with
reclaimed water via IPR, in rivers heavily impacted by existing de facto reuse practices. It
provides important evidence for water resource managers, when considering the suitability
and feasibility of alternative water supply options. The results presented could also be
important for informing the public of the potential benefits of IPR. This evidence has the
potential to help improve public perception of such reuse schemes and reduce public
opposition.

Acknowledgements

The authors would like to thank South East Water Ltd for funding this research. The authors
would also like to thank Southern Water Services (UK) for providing access to treated
wastewater samples.

References

of biochemical fingerprinting data from multi-catchments. Journal of Applied Microbiology,

enterococci. Part 2: Membrane filtration method. International Organisation for
Standardisation, Geneva, Switzerland.


tool for viral waterborne disease control. Environmental Science and Technology, 46 (2),

Environment Protection and Heritage Council (EPHC), the National Health and Medical
Research Council (NHMRC), the Natural Resource Management Ministerial Council
treatment. https://eur-lex.europa.eu/legal-
action in the field of water policy. https://eur-lex.europa.eu/legal-
EU (European Union) 2006. Directive 2006/7/EC. Concerning the management of bathing
Field, G. K. Samadpour, M. (2007). Fecal source tracking, the indicator paradigm, and
managing water quality. Water Research. 41 (16), 3517–3538. PMID: 17643471,
10.1016/j.watres.2007.06.056.


Payment P, Locas A. 2010. Pathogens in Water: Value and Limits of Correlation with Microbial Indicators. Groundwater, 49 (1), 4-11. PMID: 20477877, 0.1111/j.1745-6584.2010.00710.x


Table 1. Identified hazard organisms chosen as representative of viral, bacterial and protozoal pathogens (Woodall, 2009)

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Size (µm)</th>
<th>Disease/Illness</th>
<th>Transmission</th>
<th>Infectious Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>0.07-0.1</td>
<td>Respiratory, eye and throat infections and gastroenteritis.</td>
<td>Water, aerosols</td>
<td>Low infectious dose. Healthy individuals less affected.</td>
</tr>
</tbody>
</table>
| Salmonella    | 0.2-2.0   | Gastroenteritis with fever, cramps and diarrhoea.       | Water and Food    | Varies based on individual at risk - age, health, etc.
| Cryptosporidium | 4.0-10.0 | Diarrhoea, nausea, vomiting Water and fever. | One oocyst could cause illness. |

Table 2. Selected parameters for dose response models for adenovirus, Salmonella and Cryptosporidium pathogens (CAMRA, 2013)

<table>
<thead>
<tr>
<th>Identified strain</th>
<th>Host type</th>
<th>Best fit model</th>
<th>Optimised parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>Human</td>
<td>Exponential</td>
<td>K=6.07E-01</td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>Human</td>
<td>Exponential</td>
<td>K=5.72E-02</td>
</tr>
<tr>
<td>Salmonella</td>
<td>Human</td>
<td>Beta-Poisson</td>
<td>α=1.75E-01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N=1.11E+061</td>
</tr>
</tbody>
</table>

Table 3. Spearman’s Rank correlation values between faecal indicators and pathogenic organisms

<table>
<thead>
<tr>
<th>Pathogenic organisms (log copies/L)</th>
<th>Faecal indicator organisms (log CFU or PFU/L)</th>
<th>N</th>
<th>Correlation</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>Somatic coliphages</td>
<td>44</td>
<td>0.426</td>
<td>0.004</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>GB124 phages</td>
<td>44</td>
<td>0.252</td>
<td>0.099</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Intestinal enterococci</td>
<td>44</td>
<td>0.153</td>
<td>0.320</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Thermotolerant coliforms</td>
<td>44</td>
<td>0.214</td>
<td>0.162</td>
</tr>
<tr>
<td>Salmonella</td>
<td>Somatic coliphages</td>
<td>44</td>
<td>-0.124</td>
<td>0.421</td>
</tr>
<tr>
<td>Salmonella</td>
<td>GB124 phages</td>
<td>44</td>
<td>0.230</td>
<td>0.133</td>
</tr>
<tr>
<td>Salmonella</td>
<td>Intestinal enterococci</td>
<td>44</td>
<td>0.118</td>
<td>0.446</td>
</tr>
<tr>
<td>Salmonella</td>
<td>Thermotolerant coliforms</td>
<td>44</td>
<td>0.114</td>
<td>0.462</td>
</tr>
</tbody>
</table>
Figure 1. River water quality sites and wastewater reuse augmentation location schematic map.
Figure 2. Concentrations of faecal indicator organisms at river sites and in treated wastewater. Outliers (observations>1.5 times the interquartile range) are represented by a *. Boxes represent the interquartile range (n=44). W = Conventionally treated wastewater from the coastal wastewater treatment works; X = Drinking water abstraction river site; Y = Proposed reclaimed water augmentation site 1.5 km upstream of the drinking water abstraction site; Z = Proposed reclaimed water augmentation site 3 km upstream of the drinking water abstraction site.
Figure 3. Concentrations of pathogens at river sites and in treated wastewater. Outliers (observations>1.5 times the interquartile range) are represented by a *. Boxes represent the interquartile range (n=44). W = Conventionally treated wastewater from the coastal wastewater treatment works; X = Drinking water abstraction river site; Y = Proposed reclaimed water augmentation site 1.5 km upstream of the drinking water abstraction site; Z = Proposed reclaimed water augmentation site 3 km upstream of the drinking water abstraction site;
Figure 4. Mean probability of adenovirus infection per single event for swimming, canoeing, rowing, fishing and kayaking at site X (drinking water abstraction river site) after augmentation with conventionally treated wastewater. Risk of infection was calculated stochastically using @Risk Monte Carlo simulation (10000 iterations).
Figure 5. Mean probability of *Cryptosporidium* infection per single event for swimming, canoeing, rowing, fishing and kayaking at site X (drinking water abstraction river site) after augmentation with conventionally treated wastewater. Risk of infection was calculated stochastically using @Risk Monte Carlo simulation (10000 iterations).
**Figure 6.** Mean probability of *Cryptosporidium* infection annually for swimming, canoeing, rowing, fishing and kayaking at site X (drinking water abstraction river site) after augmentation with conventionally treated wastewater. Risk of infection was calculated stochastically using @Risk Monte Carlo simulation (10000 iterations).
Figure 7. Mean probability of adenovirus infection per single event for swimming, canoeing, rowing, fishing and kayaking at site X (drinking water abstraction river site) after augmentation with IPR reclaimed water. Risk of infection was calculated stochastically using @Risk Monte Carlo simulation (10000 iterations).