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

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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. Fourty-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

75 can provide storage, transport and may act as an additional barrier for the protection of public
76 health (U.S. EPA, 2012).
77
78 Significant global population increases and projections for water availability indicate that
79 water deficits will become an increasing problem worldwide and wastewater discharge
80 volumes will increase. For example, projections for water availability for the UK indicate that
81 even under lower bound scenarios (a low population and medium climate change projection)
82 there will be significant water deficits in the south-east of England and elsewhere by 2050
83 (HR Wallingford, 2015). Projected water deficits have meant that water managers must
84 investigate alternative non-traditional sources, with which to augment water supplies. IPR
85 represents a promising alternative water source, providing a sustainable supply and a
86 potential reduction of pollutant release into the environment. Whilst it is recognised that
87 advanced treatment technology makes it possible to treat wastewater (even for direct potable
88 reuse (DPR)) to the standard of intended use (US EPA, 2012), it is also important to limit
89 where possible the associated costs, energy consumption and carbon output, as this has
90 implications for the viability and sustainability of a proposed scheme.
91
92 River catchments are typically used throughout the year by a range of stakeholders and end-
93 users for many different (sometimes conflicting) uses including recreational activities, such
94 as swimming and kayaking. Experience has shown that public opposition can be a significant
95 barrier to the successful implementation of wastewater reuse schemes (Hurlimann and
96 Dolnicar, 2009; Fielding, Dolnicar and Schultz, 2019) and customer satisfaction is an
97 important consideration for water companies. Therefore, in order to make informed decisions
98 considering the introduction of wastewater reuse schemes and to alleviate public health
99 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

125 public for recreational pursuits (including swimming and boating). The combination of
126 different uses within inland surface waters makes the investigation of human health risks to
127 recreational users from existing *de facto* reuse activities critical. In addition, it is important to
128 predict the impact of the introduction of IPR schemes on the existing human health risk, as
129 water resource managers search for alternative water sources. Therefore, the aim of this
130 research was to quantify reference bacterial, viral and protozoan pathogens of human health
131 concern and to use a QMRA approach to calculate the probability of potential pathogen
132 infection for recreational users under: 1) *de facto* reuse conditions; 2) after additional
133 augmentation with conventionally treated wastewater; 3) after additional augmentation with
134 reclaimed wastewater subjected to advanced treatment through planned IPR schemes. There
135 is currently very limited evidence in the literature, of the impact of *de facto* and IPR
136 augmentation on surface water quality and microbial risk to the public through recreation. *De*
137 *facto* reuse is a common practise globally and IPR represents an important alternative water
138 source for many countries. Therefore, the findings of this research contribute important
139 empirical evidence of the risk to recreational users in scenarios relevant worldwide. As such,
140 empirical data from this research will help water resource managers to make informed
141 decisions on whether to include and select IPR schemes as options for the provision of
142 alternative water sources in water management plans. The evidence presented could also be
143 used to inform the public of the benefits of such reuse schemes, increasing public acceptance
144 through better understanding of the processes and risks involved.
145
146 **Methods**
147 ***Monitoring programme.***
148 Thirty-three surface water samples (31 L each time) were collected at a drinking water
149 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 Multifloä lamella clarifiers and then secondary treatment through Biostyrä 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).

175

176 ***Quantification of faecal indicator organisms.***

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

187

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

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

192

193 ***Concentration for detection of pathogenic viruses.***

194 Ten litre samples were concentrated for human adenovirus (AdV type F and G) using a
195 skimmed milk flocculation procedure, as described in Purnell et al. (2016) and Calgua et al.
196 (2013). Before sample concentration, conductivity was measured and altered to achieve
197 levels greater than 1.5 µS/cm using sterile artificial sea salts (Sigma-Aldrich, UK). The pH of
198 the sample was also reduced to 3.5 by adding HCL 1 N. Once the samples were prepared, a
199 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 oC.

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 ºC until further analysis (within 4 months of freezing).

225

226 ***Quantitative real-time PCR assays.***

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

233

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

235 This QMRA included four principal steps: (1) hazard identification, (2) exposure assessment,
236 (3) effect assessment (dose-response) and (4) risk characterisation. Potential pathogen
237 infection risk for the public using the river sites for recreational activities was calculated
238 under existing *de facto* wastewater reuse conditions (scenario 1). These results were then
239 compared to risk calculations for two potential future scenarios: 2) river water augmentation
240 with conventionally treated wastewater from the coastal wastewater treatment works (to
241 simulate future increases in *de facto* wastewater discharge associated with increasing
242 population) and 3) river water augmentation with reclaimed water after further treatment
243 through the proposed water reclamation facility. Scenarios 2 and 3 were calculated for a
244 range of augmentation scenarios (assuming augmentation proportions of 0, 10, 20, 30, 40 and
245 50% of the total river flow). In summer months during 2016 and 2017 mean flows at the river
246 sites were 37% below mean winter flow rates. The minimum flow rate recorded was 86% less
247 than mean winter flow rates. Therefore, proposed augmentation scenarios fall within a
248 realistic range for this catchment.

249

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: $P(response) = 1 - \exp(-k\ x\ dose)$

Beta – Poisson model: $1 - \left[1 + dose\ \frac{\left(2^{\frac{1}{\alpha}} - 1\right)}{N_{50}}\right]^{-\alpha}$

299 Where exp = exponential, dose = calculated exposure dose, N_{50} represents the dose at which
300 50% of the population is expected to be affected, Values for k represent the survival
301 probabilities.
302
303 ***Risk characterisation.***
304 Risk characterisation combines the information from exposure assessment and effect
305 assessment to determine the probability of infection per person per year. This was
306 stochastically estimated using the software @Risk version 7.5.1 (Palisade Corporation). Risk
307 of infection was calculated for a single event (an activity untaken once) and annually
308 assuming that an individual would partake in the select activity once per month.
309
310 ***Statistical analysis.***
311 All data distributions for parameters were analysed for normality. Non-parametric statistical
312 tests were used because the data were not normally distributed. To determine if there were
313 statistically significant differences between the water quality of the river sites (Sites X, Y and
314 Z), Kruskal-Wallis statistical tests were used. For comparison between river site data and
315 treated wastewater data the Mann-Whitney test was used. Correlation analysis was performed
316 using the Spearman's Rank correlation coefficient. The statistical tests described were
317 conducted with the statistical software Minitab version 19 with a significance level set a 5%.
318 The results of statistical tests are presented in brackets with the P value result to support the
319 interpretation within the text.
320
321 **Results**
322 ***Monitoring of faecal indicator organisms.***

323 Figure 2 presents the concentrations of faecal indicator organisms at river sites and in treated
324 wastewater (intestinal enterococci, faecal coliforms, somatic coliphages, and human-specific
325 bacteriophage capable of infecting *Bacteroides* host strain GB124) between 25th July 2016
326 and the 15th May 2017. Concentrations of faecal indicator organisms were compared across
327 the river sites (the drinking water abstraction site (site X) and proposed reclaimed wastewater
328 augmentation sites (site Y – 1.5 km upstream and site Z – 3 km upstream of the drinking
329 water abstraction site). Statistically there was no significant difference between the
330 concentrations of intestinal enterococci, somatic coliphages and phages infecting human-
331 specific *Bacteroides* strain GB124 between the river sites (Kruskal-Wallis: P-value = 0.152,
332 0.907 and 0.577, respectively). Whilst there was a significant difference between
333 concentrations of thermotolerant coliforms across the river sites (Kruskal-Wallis; P-value
334 =0.034), the observed difference in concentrations of 0.58 log CFU/ml was relatively small.
335 The faecal indicator organism concentrations in river sites were then compared to
336 concentrations in treated wastewater. Data from all river water samples were grouped
337 together, since differences between the sites were insignificant. Concentrations of intestinal
338 enterococci, thermotolerant coliforms, somatic coliphages and phages infecting human-
339 specific GB124 were significantly higher in treated wastewater (Mann-Whitney; P-value =
340 0.00, 0.00, 0.02 and 0.00, respectively). Median concentrations of intestinal enterococci were
341 between 1.74 and 1.92 log CFU/ml greater and thermotolerant coliforms 1.7 and 2.28 log
342 CFU/ml greater in treated wastewater, compared with river water samples. Median
343 concentrations of somatic coliphages were also between 1.30 and 1.44 log PFU/ml greater in
344 treated wastewater. Concentrations of phages infecting human-specific GB124 were low in
345 river water samples, with a median of <0.01 log PFU/ml, whereas the median concentration
346 in treated wastewater was 0.78 log PFU/ml.
347

348 ***Pathogens.***

349 Figure 3 presents the concentrations of pathogens (adenovirus, *Salmonella* and
350 *Cryptosporidium*) at river sites and in treated wastewater. . There was no statistically
351 significant difference in concentrations of adenovirus or *Salmonella* between the river sites
352 (Kruskal-Wallis; P-value = 0.39 and 0.970, respectively). Concentrations of *Salmonella* were
353 not significantly different between river sites and treated wastewater (Mann-Whitney; P-
354 value = 0.75), with relatively low concentrations in both river water samples and treated
355 wastewater (medians both <0.01 log copies/L). According to statistical analysis,
356 concentrations of adenovirus were significantly higher in treated wastewater (Mann-Whitney;
357 P-value = 0.04), with concentrations between 0.04 and 0.73 log copies/L higher. However, it
358 is noteworthy that median concentrations of adenovirus at one of the proposed augmentation
359 sites (Site Z - 3km upstream of the drinking water abstraction site) were only 0.04 log
360 copies/L lower than in the treated wastewater. *Cryptosporidium* was only detected in a single
361 sample from treated wastewater (n=11) at 2.10 log copies/ L (October 17th, 2016) and not in
362 river water samples tested (n= 33).

363

364 ***Correlation between indicator organisms and pathogens.***

365 The Spearman's Rank correlation coefficient was used to determine the significance and
366 strength of correlation between the indicator and pathogenic organisms monitored within this
367 study (Table 3). Results demonstrated that only somatic coliphages correlated with
368 adenoviruses. A significant moderate-strength positive (r_2= 0.426) correlation was observed
369 between these organisms (p-value of 0.004). No other faecal indicator organism correlated
370 with *Salmonella* or adenovirus and somatic coliphages did not correlate with *Salmonella*.
371 *Cryptosporidium* was not included in correlation analysis, because there was only a single
372 detection in treated wastewater from the coastal wastewater treatment works.

373

374 ***Quantitative microbial risk assessment***

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

384

385 ***Scenario 1: existing infection risk to recreational users.***

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

395

396 ***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 C*ryptosporidium*. 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

497 pathogenic organisms, respectively. However, these findings do imply that alternative
498 indicators such as bacteriophages should be considered for determining the risk of viral
499 pathogen infection. This is supported by correlation analysis that found a significant positive
500 correlation between somatic coliphages and adenovirus in this study (p-value =0.004; r_2=
501 0.426). Increasing recognition of the limitations of FIB to predict viral pathogens has led to
502 the growing use of coliphages (bacteriophages that infect *Escherichia coli*) in water
503 regulation. As an example, the United States Environmental Protection Agency (USEPA) has
504 reviewed and is exploring the introduction of coliphages as possible indicators of faecal
505 contamination for surface water quality (USEPA, 2015).
506
507 For *Salmonella* the risk of infection under all scenarios was shown to be negligible.
508 *Salmonella* is a bacterial pathogen and is therefore likely to inactivate more readily through
509 wastewater treatment and in the environment, which could explain the lower concentrations
510 detected in this research in comparison to adenovirus (Moce-Llivina et al., 2005).
511 *Cryptosporidium* was only detected in treated wastewater. Wastewater often contains human
512 and non-human animal faecal sources from livestock and wildlife (including rodents in the
513 wastewater network and birds at wastewater treatment sites). So, whilst *Cryptosporidium* is a
514 zoonotic pathogen, its presence in treated wastewater is not unusual. Zahedi et al (2018)
515 summarises the results of 27 studies (including the results of their study) that assess the
516 prevalence of *Cryptosporidium* in wastewater. These studies conducted across Africa, Asia,
517 Australia, Europe, North America and South America observed *Cryptosporidium* prevalence
518 in wastewater ranging from 6.4-100%. QMRA scenario 2 (after augmentation with
519 conventionally treated wastewater) indicated an increased risk of infection with
520 *Cryptosporidium*. Whilst the risk remains moderate, the introduction of any treated
521 wastewater has the potential to introduce a risk that was not previously present according to

522 our monitoring results (in 10L samples). Low concentrations of *Cryptosporidium* are still a
523 concern as infectivity studies and dose-response modelling have shown that single oocyst
524 infection probabilities can be up to 72% (Messner and Berger, 2016). *Cryptosporidium* is
525 resistant to chlorination commonplace in conventional treatment works and requires an
526 alternative treatment for removal, such as filtration and secondary disinfection with UV light
527 or ozone (Betancourt and Rose, 2004; Hijnen et al., 2006; Zhang and Farahbakhsh, 2007;
528 Chaudhry et al., 2015; Purnell et al., 2016; Chalmers et al., 2019). In contrast, the model
529 suggests that the risk of infection from adenovirus under existing *de facto* reuse conditions
530 was much higher (≥ 0.90) and did not increase substantially with augmentation with
531 additional conventionally treated wastewater. Viral and protozoan micro-organisms are
532 known to be more resistant to common wastewater treatment processes and the data
533 presented here supports this understanding. The findings presented here indicate that
534 advanced treatment to remove these organisms would be required to avoid further
535 deterioration of water quality at river sites (X, Y and Z). The QMRA model results suggest
536 that the treatment trains proposed for the water reclamation facility, would produce a far
537 superior microbiological water quality than is currently observed at the river sites featured in
538 this study. Discharge of this quality of water, would act to dilute existing pathogens in the
539 river and reduce the risk of infection to recreational users. The planned water reclamation
540 facility also adheres to current international standards for wastewater reuse for the purposes
541 of augmentation, that require the absence of coliphages (viral indicators) and
542 *Cryptosporidium* (U.S. EPA, 2012; EPHC, 2008; UKWIR, 2005). Research from Chaudhry
543 et al. (2017) has shown similar outcomes when modelling the infection risks associated with
544 the blending of *de facto* reuse waters with direct potable reuse (DPR) waters (finished
545 product). Their results indicated that risk was affected mostly by contamination levels in
546 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.

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769 **Table 1**. Identified hazard organisms chosen as representative of viral, bacterial and

770 protozoal pathogens (Woodall, 2009)

Pathogen	Size (μm)	Disease/Illness	Transmission	Infectious Dose
Adenovirus	0.07-0.1	Respiratory, eye and throat infections and gastroenteritis.	Water, aerosols	Low infectious dose. Healthy individuals less affected.
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 and fever.	Water	One oocyst could cause illness.

771

772 **Table 2**. Selected parameters for dose response models for adenovirus, *Salmonella* and

773 *Cryptosporidium* pathogens (CAMRA, 2013)

774

Identified strain	Host type	Best fit model	Optimised parameters
Adenovirus	Human	Exponential	K=6.07E-01
Cryptosporidium	Human	Exponential	K=5.72E-02
Salmonella	Human	Beta-Poisson	α=1.75E-01 N_{50}=1.11E+061

775

776 **Table 3.** Spearman's Rank correlation values between faecal indicators and pathogenic

777 organisms

Pathogenic organisms (log copies/L)	Faecal indicator organisms (log CFU or PFU/L)	N	Correlation	P-Value
Adenovirus	Somatic coliphages	44	0.426	0.004
Adenovirus	GB124 phages	44	0.252	0.099
Adenovirus	Intestinal enterococci	44	0.153	0.320
Adenovirus	Thermotolerant coliforms	44	0.214	0.162
Salmonella	Somatic coliphages	44	-0.124	0.421
Salmonella	GB124 phages	44	0.230	0.133
Salmonella	Intestinal enterococci	44	0.118	0.446
Salmonella	Thermotolerant coliforms	44	0.114	0.462

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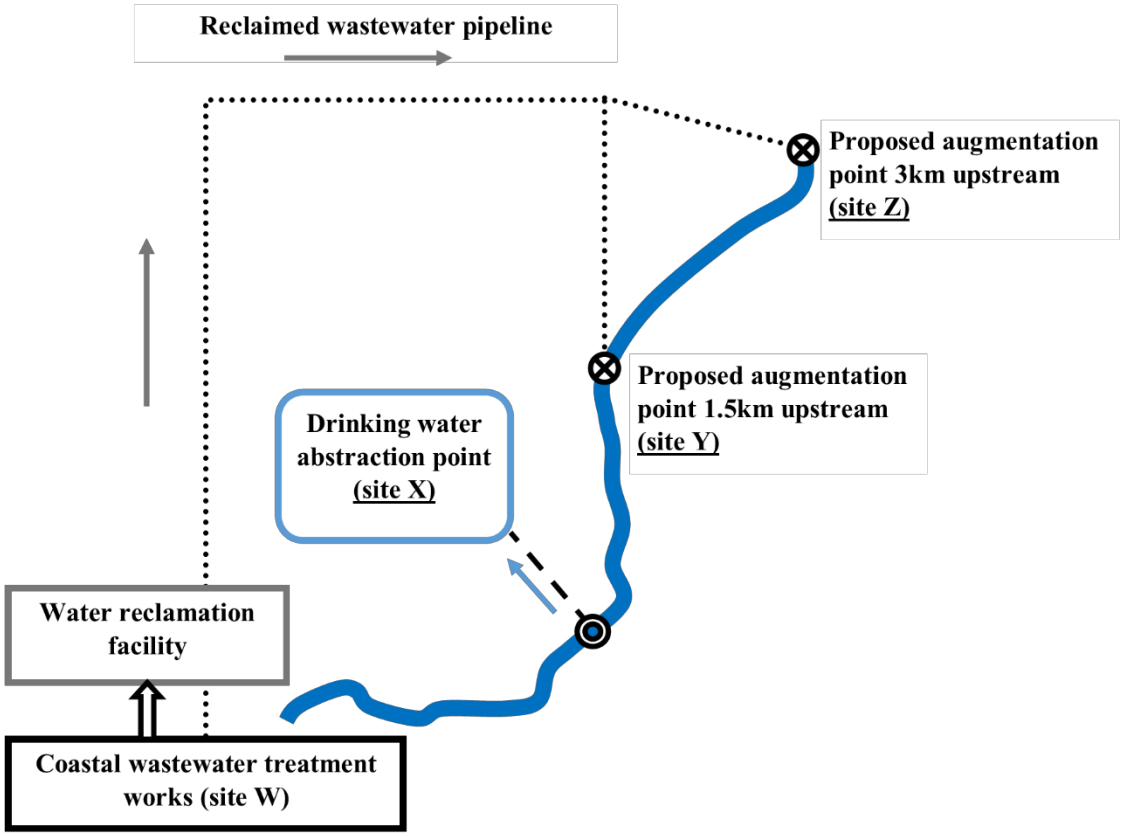
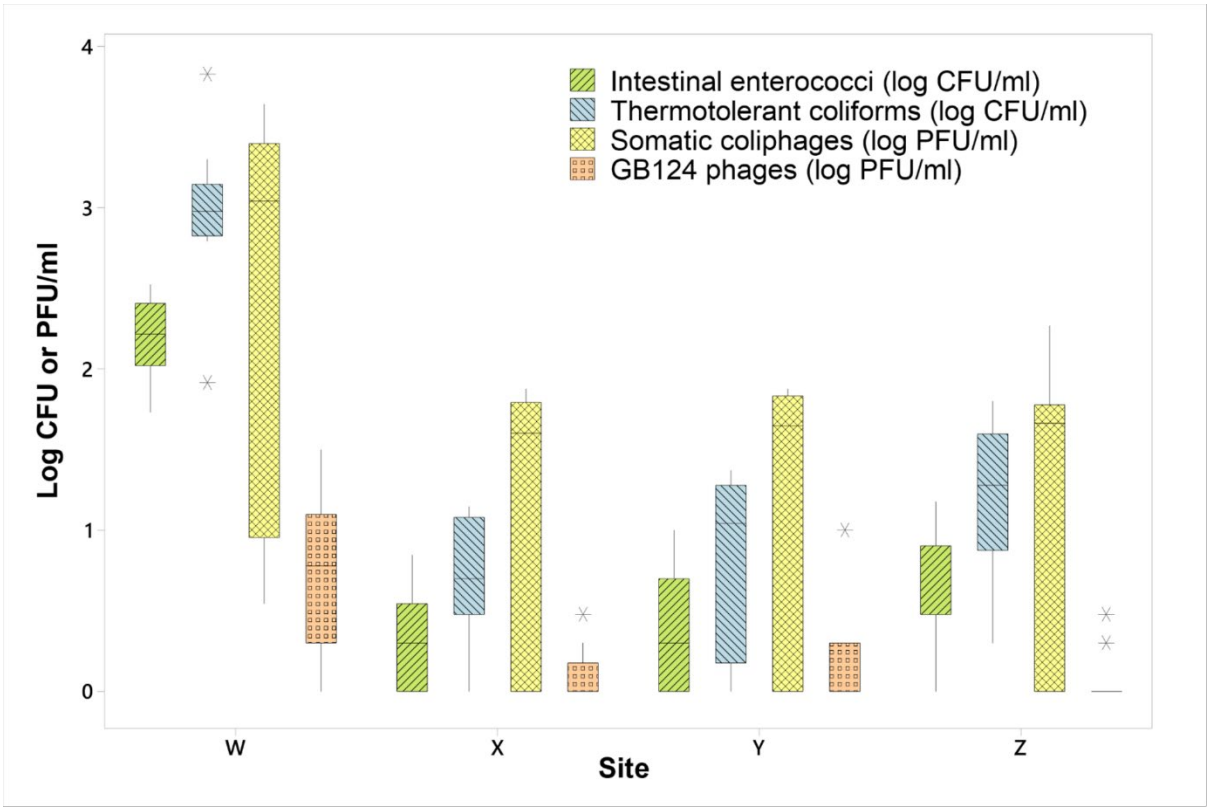


779

780 **Figure 1.** River water quality sites and wastewater reuse augmentation location schematic

781 map.

782



783

784 **Figure 2**. Concentrations of faecal indicator organisms at river sites and in treated
785 wastewater. Outliers (observations>1.5 times the interquartile range) are represented by a *.
786 Boxes represent the interquartile range (n=44). W = Conventionally treated wastewater from
787 the coastal wastewater treatment works; X = Drinking water abstraction river site; Y =
788 Proposed reclaimed water augmentation site 1.5 km upstream of the drinking water
789 abstraction site; Z = Proposed reclaimed water augmentation site 3 km upstream of the
790 drinking water abstraction site.

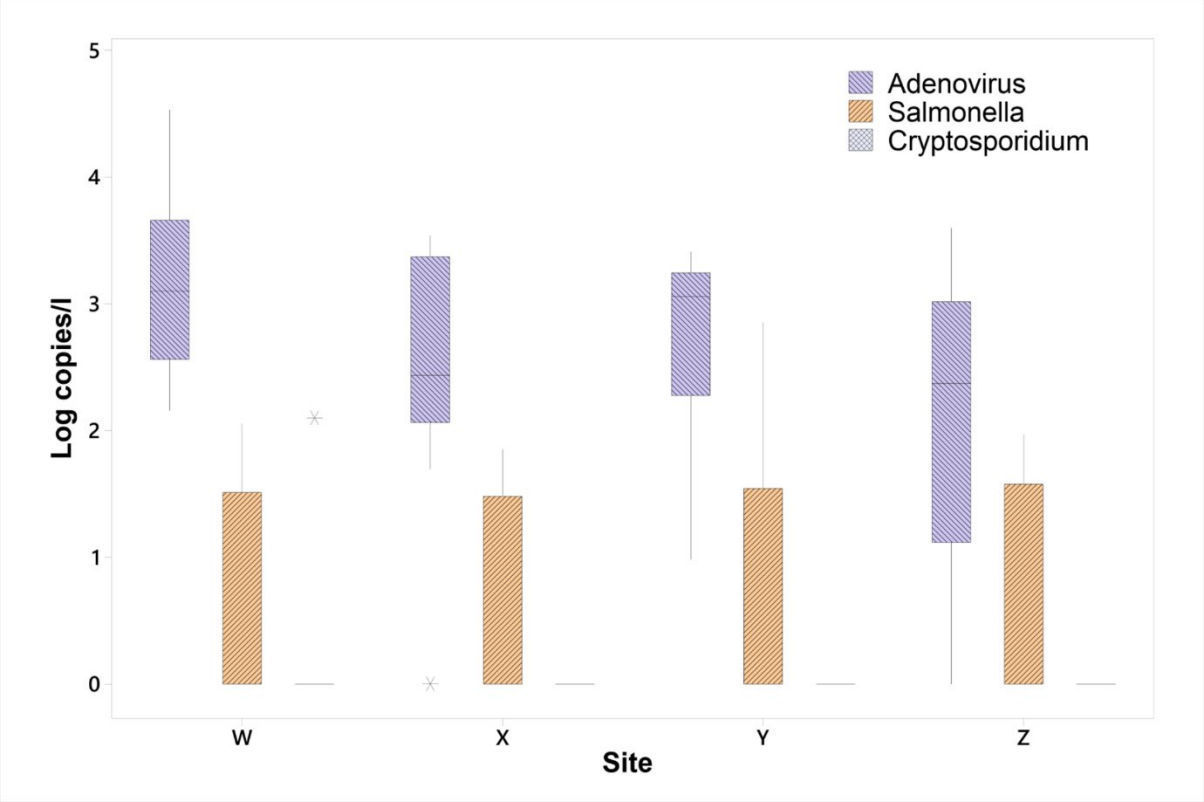


Figure 3. Concentrations of pathogens at river sites and in treated wastewater. Outliers (observations>1.5 times the interquartile range) are represented by a *. Boxes represent the interquartile range (n=44). W = Conventionally treated wastewater from the coastal wastewater treatment works; X = Drinking 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;

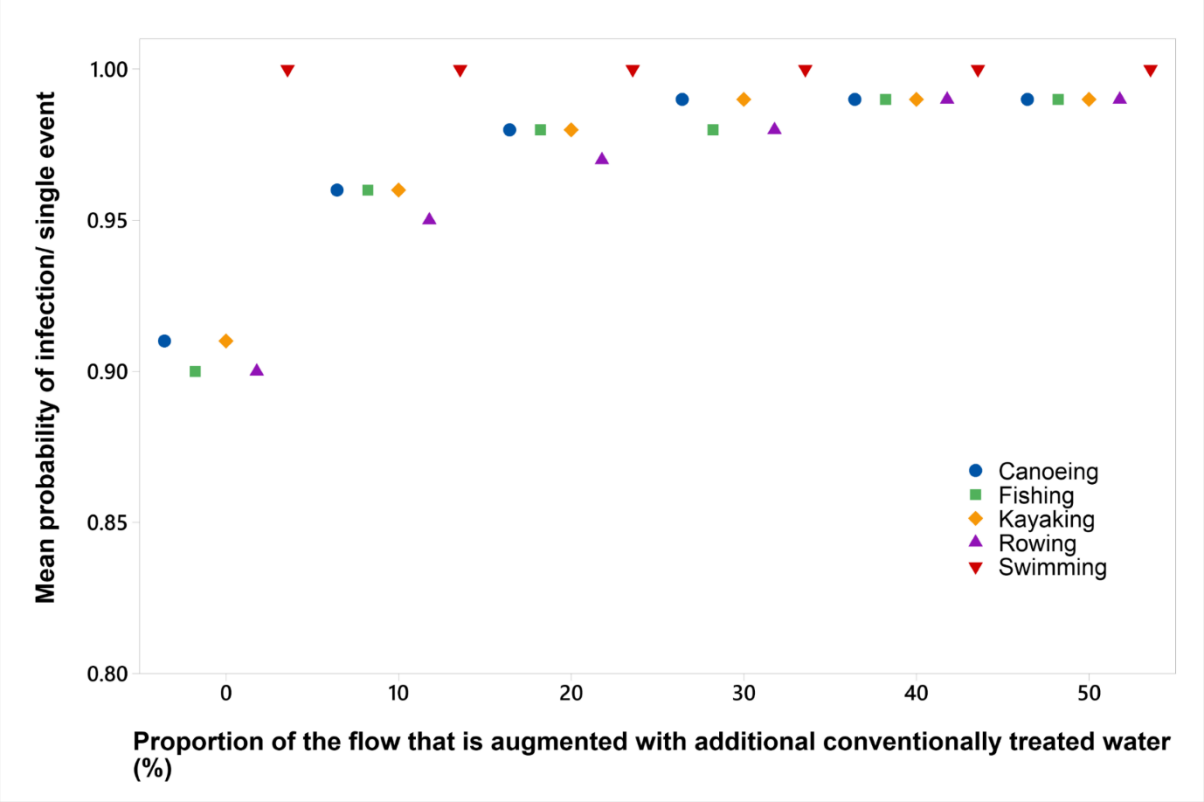
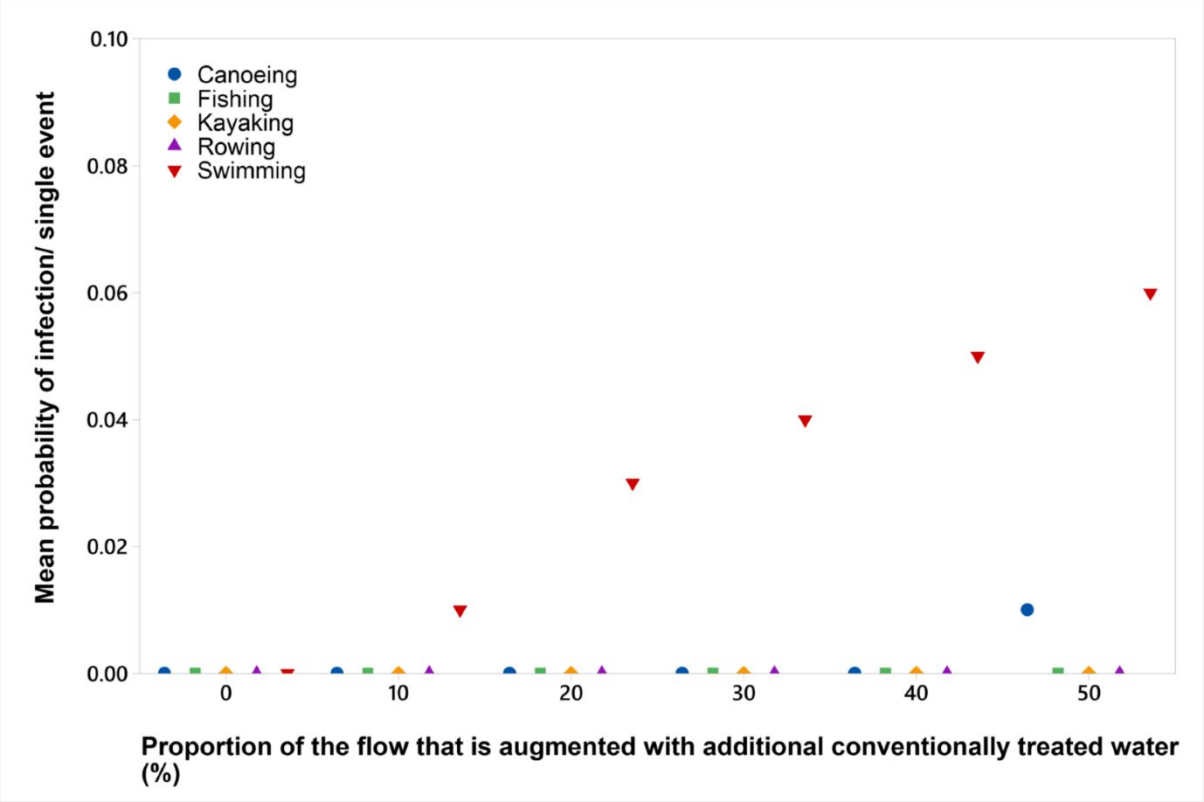


Figure 4. Mean probability of adenovirus infection per single event for swimming, canoeing, rowing, fishing and kayaking at site X (drinking water abstraction river site) after augmentation with conventionally treated wastewater. Risk of infection was calculated stochastically using @Risk Monte Carlo simulation (10000 iterations).



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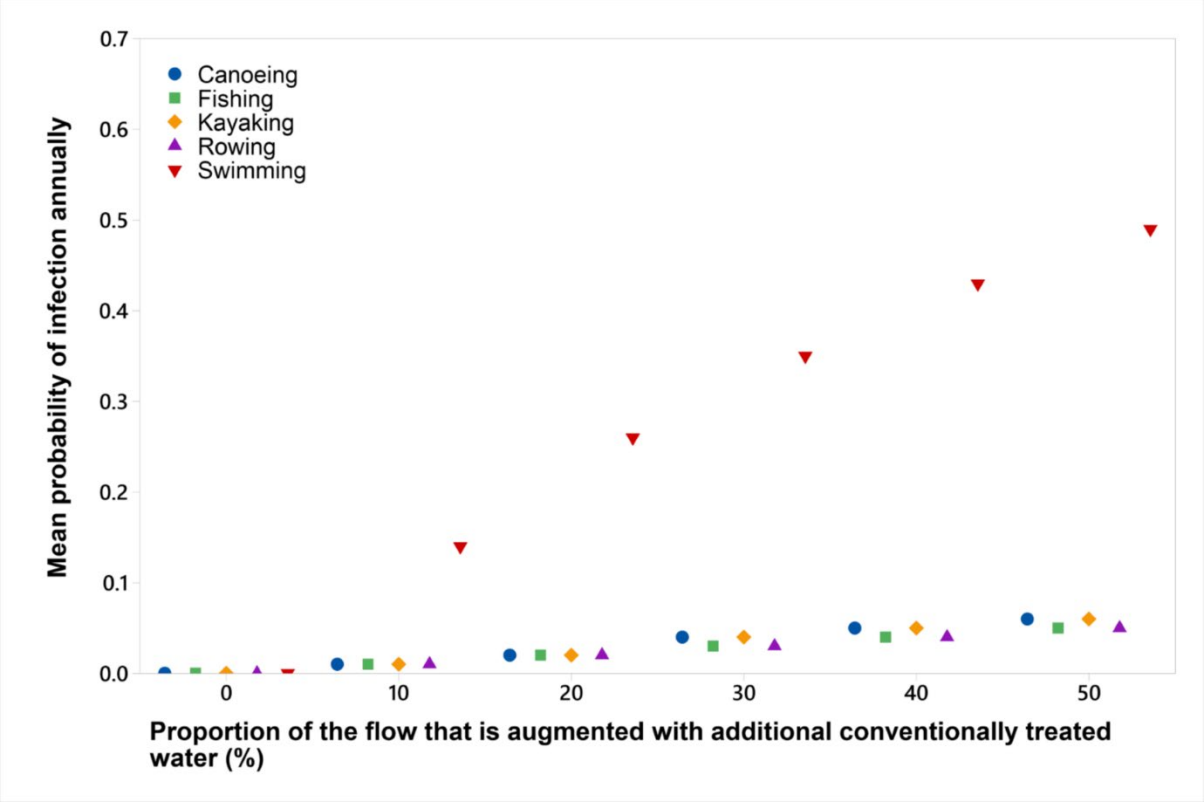
805 **Figure 5**. Mean probability of *Cryptosporidium* infection per single event for swimming,
806 canoeing, rowing, fishing and kayaking at site X (drinking water abstraction river site) after
807 augmentation with conventionally treated wastewater. Risk of infection was calculated
808 stochastically using @Risk Monte Carlo simulation (10000 iterations).



809

810 **Figure 6.** Mean probability of *Cryptosporidium* infection annually for swimming, canoeing,
811 rowing, fishing and kayaking at site X (drinking water abstraction river site) after
812 augmentation with conventionally treated wastewater. Risk of infection was calculated
813 stochastically using @Risk Monte Carlo simulation (10000 iterations).

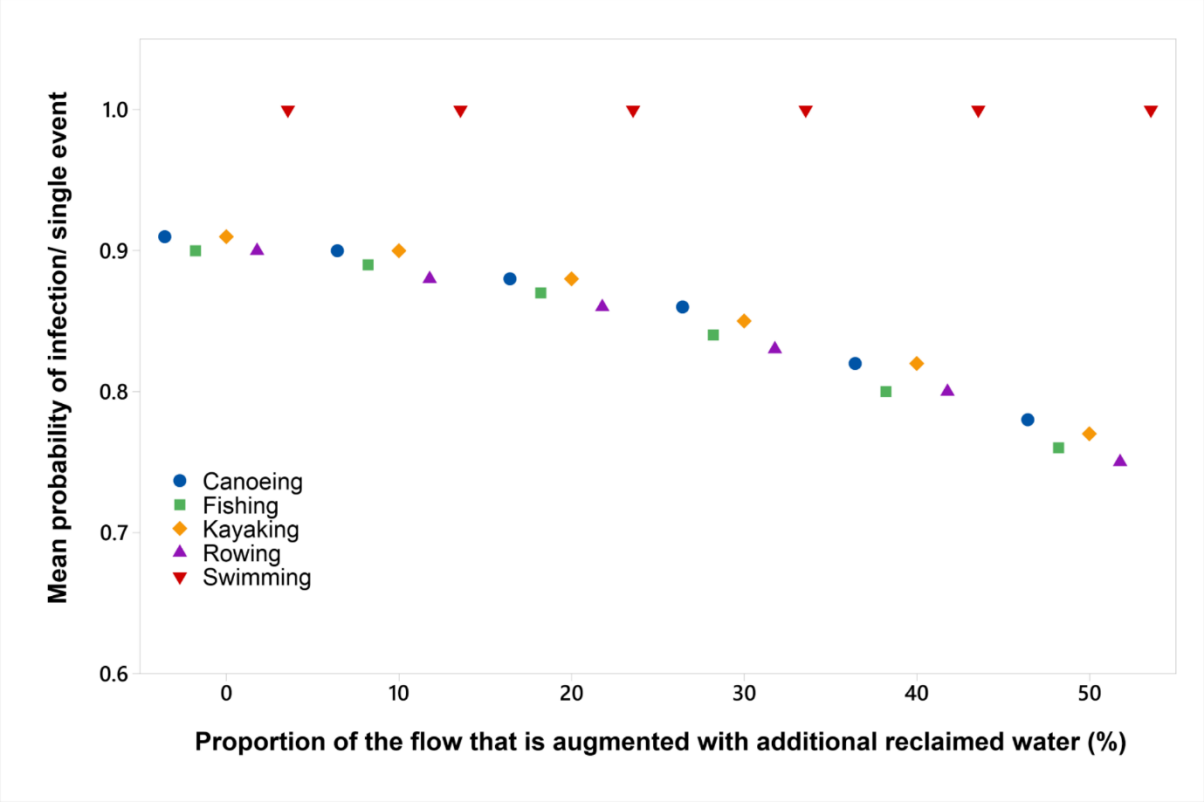


Figure 7. Mean probability of adenovirus infection per single event for swimming, canoeing, rowing, fishing and kayaking at site X (drinking water abstraction river site) after augmentation with IPR reclaimed water. Risk of infection was calculated stochastically using @Risk Monte Carlo simulation (10000 iterations).