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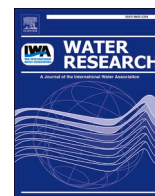
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# Benthic sediment as stores and sources of bacteria and viruses in streams: A comparison of baseflow vs. stormflow longitudinal transport and residence times

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## ABSTRACT

The presence of bacteria and viruses in freshwater represents a global health risk. The substantial spatial and temporal variability of microbes leads to difficulties in quantifying the risks associated with their presence in freshwater. Fine particles, including bacteria and viruses are transported and accumulated into shallow streambed (i.e., benthic) sediment, delaying the downstream transmission during baseflow conditions but contributing to their resuspension and transport downstream during stormflow events. Direct measurements of pathogen accumulation in benthic sediments are rare. Until now, the dynamic role of benthic sediment as both a store and source of microbes, has not been quantified. In this study, we analyze microbial abundance in benthic sediment along a 1 km reach of an intermittent Mediterranean stream receiving inputs from the effluent of a wastewater treatment plant, a known point source of microbes in streams. We sampled benthic sediment during a summer drought when the wastewater effluent constituted 100 % of the stream flow, and thus, large accumulation and persistence of pathogens along the streambed was expected. We measured the abundance of total bacteria, *Escherichia coli* (as a fecal indicator), and presence of enteric rotavirus (RoV) and norovirus (NoV). The abundance of *E. coli*, based on qPCR detection, was high ( $4.99 \cdot 10^2$  gc /cm<sup>2</sup>) along the first 100 m downstream of the wastewater effluent input and in general decreased with distance from the source, with presence of RoV and NoV along the study reach. A particle tracking model was applied, that uses stream water velocity as an input, and accounts for microbial exchange into, immobilization, degradation, and resuspension out of benthic sediment during baseflow and stormflow. Rates of exchange into benthic sediment were 3 orders of magnitude higher during stormflow, but residence times were proportionately lower, resulting in increased longitudinal connectivity from up to downstream during stormflow. Model simulations demonstrated mechanistically how the rates of exchange into and out of the benthic sediment resulted in benthic sediment to act as a store during baseflow and a source during stormflow.

## 1. Introduction

Water quality of freshwaters and in particular microbial risk from pathogen presence in streams represents a challenge to global health (Boelee et al., 2019; Ramirez-Castillo et al., 2015). Surface water is

routinely monitored to assess water quality, but monthly sampling does not adequately characterize the highly variable microbial contamination loading of most streams and does not include the underlying stream sediment (Jalliffier-Verne et al., 2016). Even when surface water concentrations of fecal indicator bacteria are low, suggesting a low

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microbial health risk, counts of bacteria in the underlying sediment can be orders of magnitude higher (Jamieson et al., 2004; Mackowiak et al., 2018; Martín-Díaz et al., 2020; Petersen and Hubbart, 2020). The underlying sediment, and especially the top 3–10 cm, represents a dynamic region of the stream often referred to as the benthic zone. Benthic sediment can temporarily store pathogens and other fine particles (e.g., particulate organic matter, fine sediments, microplastics), with a wide range of processes contributing to the exchange both into and out of this region (Boano et al., 2014; Drummond et al., 2018; 2022a; Krause et al., 2022; Lewandowski et al., 2019). An important process often not considered is the exchange into and out of the benthic sediment, termed hyporheic exchange, that encompasses key transport mechanisms such as the two-way exchange of solute and fine particles from turbulence and advective transport in the near-bed region (Boano et al., 2014; Krause et al., 2011, 2017, 2022). Hyporheic exchange processes also lead to increased nutrient loading in the benthic zone, which can in turn increase the potential for pathogen growth and persistence within this zone, particularly downstream from wastewater treatment plants (Bernal et al., 2020; Castelar et al., 2022). Pathogenic bacteria and viruses are often associated with fine particulate matter, especially the smallest size fraction (Walters et al., 2014), which coincides with the particle size fraction most influenced by hyporheic flow paths in stream ecosystems (Drummond et al., 2020). This finer fraction is more mobile and more readily resuspended from benthic sediment to the water column during high flow events (Drummond et al., 2017; Filoso et al., 2015; Harvey et al., 2012; Larsen et al., 2015). In fact, fine particles can serve as a vector of pathogenic bacteria and viruses (Harrison et al., 2018; Vethaak and Leslie, 2016) and therefore their co-dependent transport mechanisms and ability to predict accumulation patterns in streams is imperative to understanding health related risk of stream ecosystems.

Pathogens in benthic sediment depend on the deposition and resuspension rates into and out of this dynamic region. During baseflow, pathogens are transported into and out of benthic sediment by hyporheic exchange processes with the potential to either be transported back to the water column or deeper into the bed for longer term storage (Drummond et al., 2015; Fluke et al., 2019; Park et al., 2017). Previous studies have shown that during baseflow, the majority of microbes and other fine particles will either deposit in the top few cm of benthic sediment (Boano et al., 2014; Drummond et al., 2014b) or transport back into the water column with a wide range of residence times leading to long-term accumulation from hours to years (Haggerty et al., 2002; Jamieson et al., 2004; Petersen and Hubbart, 2020). Since increased streamflow, particularly due to storm events, can resuspend a portion of the benthic sediment, measurements in this transient storage zone can provide detail on the potential for stream contamination following an event. In cases where benthic sediment contains high concentrations of pathogens, these stores can also represent a key source of pathogens to the surface water during higher flow events (Fluke et al., 2019; Muirhead et al., 2004; McKergow and Davies-Colley, 2010). Pathogen accumulation and potential release back to the water column is especially important in intermittent streams, with a lower capacity to dilute inputs of microbial contamination as the water levels decrease and eventually dry out during the summer months (Keller et al., 2014; Martín-Díaz et al., 2017). The inputs of wastewater treatment plant effluent into intermittent streams provide the opportunity to assess worst-case scenarios of pathogen accumulation in benthic sediment downstream of the source, especially during the period when the upstream is dry and the downstream represents 100 % of the wastewater treatment plant effluent. Therefore, we chose this time period in an intermittent stream, when the upstream was dry and only the downstream was flowing due to the wastewater effluent, to measure benthic sediment within our study reach.

The spatial and temporal variability of pathogen accumulation patterns in streams and their dependence on hydrological conditions remain largely an open question. In this context, our goal is to understand the residence times and longitudinal transport of pathogens during

baseflow and stormflow. To mathematically represent this system and appropriately characterize and predict the transient storage of pathogens in the benthic sediment, a model that can incorporate hyporheic exchange, deposition, and resuspension during both baseflow and stormflow is required. Recently, such a modeling framework was developed, based on a particle tracking mobile-immobile model (Drummond et al., 2022b). Particles representing portions of bacterial mass are partitioned into surface water, benthic sediment, and deeper sediment. Particles move downstream according to the flow in the surface water region and transition between regions at different rates that depend on flow conditions. The model was validated against *Escherichia coli* measurements, as a bacterial indicator of fecal pollution, during artificial floods and natural stormflow events (Drummond et al., 2022b). In this work, we applied this model to our study reach to explore the spatial and temporal variability of pathogen accumulation in benthic sediment during baseflow and stormflow conditions. Our study focuses on measurements of pathogenic bacteria and viruses in the benthic sediment, as this area represents the net accumulation, encompassing deposition and resuspension processes. Specifically, the model was fit to the *E. coli* benthic sediment measurements during baseflow and supported by virus presence or absence at the same locations. Our main aims were to (1) measure the potential for benthic sediment to store fecal indicator bacteria and viruses during low baseflow conditions, (2) assess if longitudinal accumulation patterns varied, and (3) assess the role of benthic sediment as stores or sources of pathogens to the stream water column by modeling spatial and temporal sediment accumulation patterns of *E. coli*. Our study quantifies and predicts the persistence of pathogens in benthic sediment, which is often unaccounted for, and improves the assessment of microbial risk in streams over a wide range of hydrological conditions.

## 2. Materials and methods

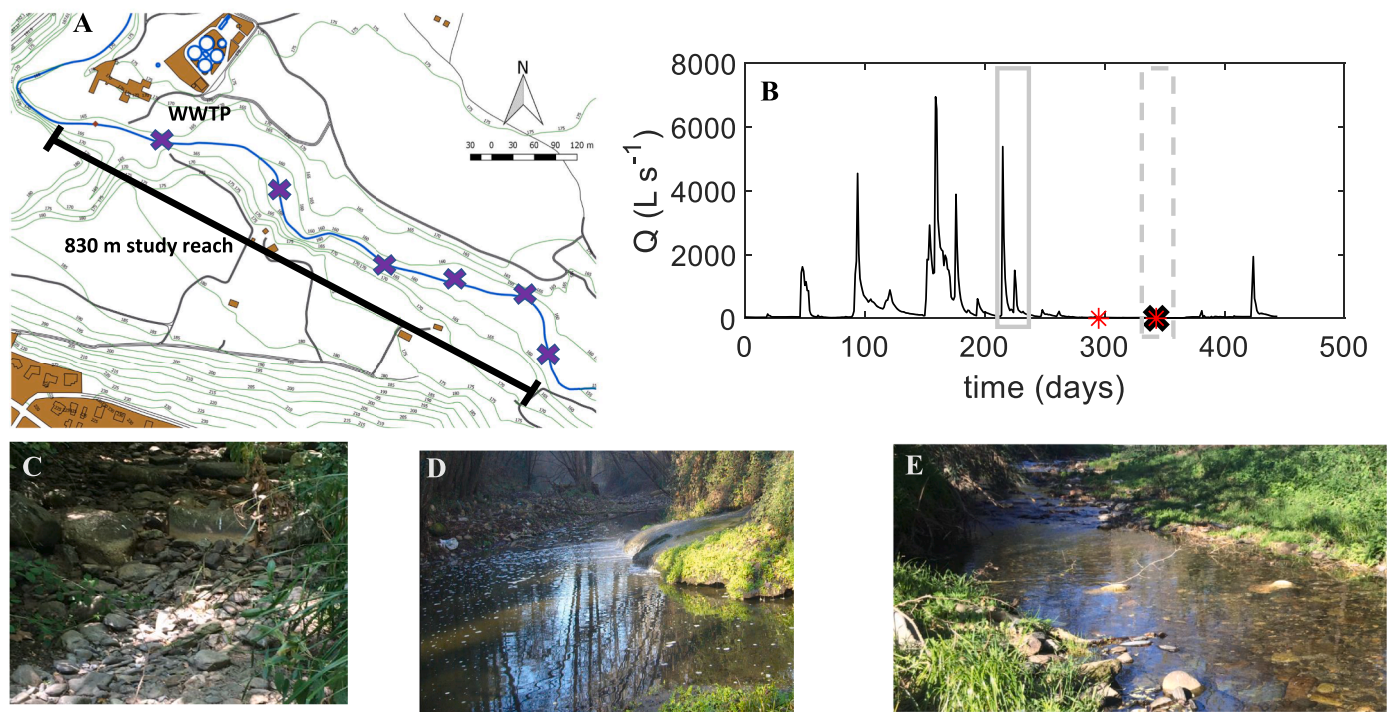
### 2.1. Description of the field study site

The field study site was located near the village of Santa Maria de Palautordera (NE Spain, lat 41°41'3.47"N, long 2°27'33.19"W) in the main course of La Tordera River, immediately downstream of a wastewater treatment plant (WWTP) outlet (Fig. 1A). The stream discharge downstream of the WWTP can vary by several orders of magnitude (Fig. 1B). Upstream of the WWTP effluent is intermittent and for a variable amount of time each year during the summer months the flow upstream decreases and eventually runs dry, as was the case on July 31, 2017 (Fig. 1C). Discharge of the WWTP is relatively constant throughout the year (mean of 27.4 L/s, Fig. 1D). Therefore, flow downstream of the WWTP is constant (Fig. 1E), with the contribution of the WWTP effluent to the total flow of the receiving stream ranging from 3 % to 100 % (Merseburger et al., 2005). Streambed substrate composition was characterized by rocks (10 %), cobbles (60 %), gravels (15 %), and fine sediment (15 %) (Bernal et al., 2020).

Samples were taken from the benthic sediment (top 3 cm) at 6 sites along a 850-m-long reach downstream of the WWTP outlet (Fig. 1A) during summer baseflow conditions on June 13, 2017 to measure presence of viruses and July 31st 2017, when flow ceased and the streambed was dry upstream of the WWTP outlet, to measure presence of both viruses and *E. coli*.

### 2.2. Field methods

On each sampling date, we collected three replicates of benthic sediment at each of the 6 downstream sampling locations. In summary, at 3 unique locations at each longitudinal transect we placed a bottom-open bucket on top of the streambed sediment, manually resuspended the top few cm, and collected a sample from the water column. Therefore, the sample was of both the water column and the benthic sediment, which is accounted for in the model fitting (Section 2.4). More



**Fig. 1.** (A) Site map with sampling locations in La Tordera River, located near the village of Santa Maria de Palautordera (modified from Drummond et al. 2022c), (B) hydrograph from August 22, 2016 - November 9, 2017, with sampling date identified with a black x for *E. coli* (July 31, 2017) and with a red asterisk for viruses (June 13 and July 31, 2017). We define a stormflow period (gray box, 140–180 days, January 9 – February 18, 2017) and a baseflow period (slotted gray box, 320–360 days, July 8 – August 17, 2017) for model projections (Section 2.4). Photographs illustrate the field site (C) upstream when there was no flow (red x in B), (D) at the continuously flowing wastewater effluent point source, and (E) downstream during low baseflow conditions.

specifically, benthic sediment was sampled by pushing a 35 cm diameter cylinder into the stream bed to form a seal and isolate the flow of the surrounding water (Drummond et al., 2022c; Meredith et al., 2021). The sampling depth was recorded (5 replicates) to calculate the volume of water within the bucket. Then, approximately the top 3 cm of sediment were agitated by hand to re-suspend the benthic sediment into the water column within the bucket. We allowed for a 10 s settling period for the majority of the sand-sized sediment to settle out of the water, such that only material less than approximately 100  $\mu\text{m}$  was sampled (Drummond et al., 2022c). A volume of stirred and well-mixed water containing suspended benthic sediment was collected using a 1 L wide-mouth Nalgene bottle. This water sample was poured into vials, without filtering, each of which was used for the analysis of a distinct variable described below (see Laboratory Methods). All samples were immediately placed on ice, protected from sunlight, and kept refrigerated at 4 °C until analyzed.

Discharge,  $Q$  [L/s], was monitored 10 times throughout the year at 200 m downstream of the WWTP point source using the cross-sectional method that consists of measurements of wetted width, water velocity ( $v$ ) and water depth across a representative cross-sectional transect (Gordon et al., 2004). We placed water level loggers (HOBO U20–001–04, Onset Corporation) in the stream to record daily measurements. Daily  $Q$  was estimated from an empirical relationship between the 10 empirical measurements and the water level on the date of the  $Q$  measurement. Daily  $Q$  was then converted to velocity ( $v$ ) by dividing by the measured water depth and average water width, that varied minimally at the measuring site throughout the year, for the sampling year (5.34 m, Fig. 1B, Drummond et al., 2022c).

### 2.3. Laboratory methods

#### 2.3.1. Viral RNA extraction and detection of rotaviruses and noroviruses by RT-qPCR as viral indicators of fecal pollution

Extraction of RNA from the benthic sediment sample was carried out using the QIAamp Viral RNA Mini Kit (QIAGEN, Chatsworth, CA, USA) following the Spin Protocol present on the QIAamp Viral RNA Mini Handbook. Briefly, 140  $\mu\text{L}$  of the benthic sediment sample was added to 560  $\mu\text{L}$  of Buffer AVL and incubated for 10 min at room temperature. 560  $\mu\text{L}$  of ethanol (96–100 %) was added. The mixture was centrifuged at 6000 X G for 1 min and the tube containing the filtrate was discarded. 500  $\mu\text{L}$  of Buffer AW1 was added and the tubes were centrifuged under the same conditions. 500  $\mu\text{L}$  of Buffer AW2 was added and the tubes were centrifuged at 20,000 X G for 3 min. The collection tubes containing the filtrate were discarded. The elution of RNA from the QIAamp column was made using 45  $\mu\text{L}$  of nuclease free water and by centrifuging for 1 min at 10,000 X G. RNA was stored at –20 °C. For each RNA isolation procedure, a negative control of isolation (NCI) was included containing only buffers.

RoV and NoV were detected using nucleic acid amplification by a one-step RT-qPCR on an ABI PRISM 7900HT sequence detection system (Applied Biosystems, USA) using an Ag Path Kit (Life Technologies) with a final volume per reaction of 10  $\mu\text{L}$  (8  $\mu\text{L}$  of master-mix and 2  $\mu\text{L}$  of sample). The qPCR assays for multiple RoV and NoV GII were used according to Gutiérrez-Aguirre et al. (2008), and Kageyama et al. (2003), respectively. The cycling conditions for RT-qPCR assays were the following: reverse transcriptase at 48 °C, 10 min; denaturation at 95 °C, 10 min; 45 cycles of denaturation at 95 °C for 15 s; and annealing/extension at 60 °C for 60 s. Reactions were run in triplicate. All the

RT-qPCR reactions were planned using GENEIO qPCR workflow application (BioSistemika LLC, Ljubljana, Slovenia) and the microplates were pipetted with the assistance of PLATR smart pipetting assistant (BioSistemika LLC, Ljubljana, Slovenia). The quantification cycle (Cq) for each reaction was obtained using the software SDS 2.4 (Applied Biosystems, CA, USA). The fluorescence thresholds were manually set for RoV and NoV according to the amplification curve. A positive control (PC) for RoV and NoV was added to monitor each amplification. A sample was considered positive when the reading provided a nonzero Cq. In addition, each positive amplification curve was manually checked and only curves showing a significant slope increase, in contrast with the negative control curves (NTC, NCI, and NCC), were considered as real positives. The detected concentrations of the two assays were close to the limit of quantification, and results are expressed as presence (+) or absence (-) of RoV and NoV.

### 2.3.2. Extraction and quantification of *E. coli* as a bacterial indicator of fecal pollution

A known volume of benthic sediment sample (~15 mL) was filtered through a 0.45  $\mu\text{m}$  nylon membrane filter (Whatman, United Kingdom). DNA was extracted using the PowerSoil DNA Isolation Kit (MO BIO, Carlsbad, CA, USA) according to the manufacturer's instructions in the PowerSoil DNA Isolation Kit Handbook. Quantification of *E. coli* was done by qPCR on an ABI PRISM 7900HT sequence detection system (Applied Biosystems, USA). The *E. coli* assay targeted the single-copy *uidA* gene as described by Frahm and Obst, using TaqMan™ Universal PCR Master Mix (Applied Biosystems, USA) with a final volume per reaction of 20  $\mu\text{L}$  (15  $\mu\text{L}$  of mastermix and 5  $\mu\text{L}$  of sample). Each sample was loaded in triplicate and PC, NTC and NCI controls were used. A standard curve with serial dilutions was prepared in triplicate from the positive control with an initial concentration of 22,698.65 gc/ $\mu\text{L}$ . The obtained standard curve was used to calculate *E. coli* concentrations as gene copies per mL (gc/mL).

To standardize the *E. coli* measurements at the different sampling locations with variable stream depths, measured concentrations (gc / mL) were converted to gene copies per stream surface area (gc /  $\text{cm}^2$ ). To do so, we multiplied the total gene copies per stream surface area by the total water volume within the sampling bucket (mL) and then dividing by the known surface area of the bottom of the bucket (0.096  $\text{m}^2$ ).

### 2.4. Particle tracking mobile-immobile model for baseflow and stormflow

To simulate microbial transport and retention in the study stream, we applied a particle tracking mobile-immobile model for in-stream transport, immobilization, and resuspension of microbes during both baseflow and stormflow conditions (Drummond et al., 2022b). The model was only fit to the *E. coli* quantitative measurements, as the virus results only provided information on presence or absence. Here, we briefly describe the specific transport processes in the model framework and parameter assumptions for both the mobile and immobile zones. A more detailed description of the model framework, including justification for the assumptions based on known transport properties of both microbes and other fine particles, is provided in Text S1. Model parameter descriptions and range in values are shown in Table S1.

The particle tracking model discretizes microbe masses into a number of Lagrangian particles subject to different processes representing microbe downstream transport and exchange between mobile and immobile zones within the stream (Fig. S1). The water column represents the mobile zone, where particles move downstream according to the time-dependent mean velocity  $v(t)$  [m/s] of the stream. The immobile zone is subdivided into the shallow benthic zone of the streambed sediment (where the samples were collected in this study) and the deeper streambed.

The mobile zone of the model framework is parameterized by  $v(t)$  according to available data, and an average velocity-dependent exchange rate into benthic sediment. This exchange from the water

**Table 1**

Presence (+) or absence (-) of Norovirus (NoV), Rotavirus (RoV) and *E. coli* from 100- 820 m downstream of the WWTP effluent input.

Distance (m):	100	300	530	623	720	820
June						
Norovirus	+	+	+	-	-	-
Rotavirus	-	-	-	-	-	-
July						
Norovirus	-	+	-	-	-	-
Rotavirus	+	-	+	+	+	-
<i>E. coli</i>	+	+	+	-	+	+

column to the underlying sediment is an important process that leads to the deposition of microbes and other fine particles with low settling velocities (Boano et al., 2014; Drummond et al., 2020). For a given value of velocity  $v$ , residence times in the water column are exponentially distributed, characterized by an instantaneous exchange rate into the benthic sediment proportional to the square of in-stream velocity (Text S1, Arnon et al., 2013; Packman et al., 2004),  $\Lambda_D(v) = c_D v^2$  [1/s], where  $c_D$  is a deposition coefficient (Table S1).

Microbes transported into the shallow benthic sediment can either be transported further into the deeper streambed or return to the water column. In the model, this is controlled by a resuspension probability  $p_R$  that can range from 0 to 1 (Table S1). During baseflow conditions, the resuspension probability takes a fixed value between 0 and 1, which is determined by fitting the model to field data. A value of 1 signifies that particles never transport into the deeper streambed, and conversely a value of 0 signifies that particles never resuspend back into the water column. Similarly to the water column, residence times in the benthic sediment are exponentially-distributed for a given velocity, with an average exchange rate back to the water column or into the deeper streambed proportional to the square of in-stream velocity (termed the resuspension rate,  $\Lambda_R(v) = c_D v^2$ , where  $c_D$  is a deposition coefficient), based on previous observations of fine sediment resuspension from the streambed (Arnon et al., 2013; Cardenas et al., 1995; Cho et al., 2010). A range of processes control the deposition and resuspension rates and therefore the retention time in the benthic zone, including irreversible filtration, porewater flow rate, and attachment to biofilms. In the model framework, the deposition and resuspension incorporate an average rate for all processes that lead to deposition and resuspension. However, a specific process can be isolated and parameterized if the data is available such as how the relationship between colloid filtration theory and a similar model framework for baseflow only showed the direct relationship by combining column experiments with in-field tracer injection studies of fine particles (Drummond et al., 2014a; Drummond et al., 2015).

The deeper streambed is characterized by a power-law residence time distribution, based on field observations of microbial retention and release from streambed sediment (Aquino et al., 2015; Drummond et al., 2014a; Haggerty et al., 2002). Compared to an exponential distribution, this represents a wider range of times over which contaminant microbes are retained in the deep streambed before being released back to the benthic sediment, where the resuspension dynamics described in the previous paragraph apply. The broadness of the distribution of retention times is controlled by an exponent  $0 < \beta < 1$  such that the probability of a retention event lasting longer than some duration  $t$  decays as  $\sim t^{-\beta}$ . The smaller the value of  $\beta$ , the broader is the distribution and the more likely are long waiting times.

During baseflow conditions, the resuspension probability takes a fixed value between 0 and 1, which is determined by fitting the model to field data. During stormflow, the same transport processes were considered in the model, but deposited particles in the benthic sediment never transport into the deep streambed and always resuspend back to the water column. This is achieved by setting  $p_R = 1$  during the rising limb of the storm hydrograph. This condition forces retained or deposited microbes already in the benthic sediment to resuspend back to the

water column instead of deeper into the streambed, aligning with field observations and previous model validation (Drummond et al., 2015, 2017;2022b; Filoso et al., 2015; Harvey et al., 2012; Lamba et al., 2015). Finally, inactivation or cell death in each zone (i.e., mobile zone, benthic zone, and deeper streambed) is represented by a first-order exponential decay and if measured and known can be easily added into the model framework. In this study, we assumed negligible decay in the water column and benthic sediment in our timescales of interest, with relatively fast transport through these zones compared to the deeper bed. Therefore, the inactivation rate in the mobile zone and benthic zone was set to 0, while the inactivation rate in the deeper streambed was fixed to  $1.4 \cdot 10^{-6} \text{ s}^{-1}$  (Drummond et al., 2014, Sinton et al., 2002).

2.4.1. Model calibration and predictions

Following the fitting procedure outlined in Drummond et al. (2019), we performed several simulations (Text S2) with parameter sets constrained to match the measurements of *E. coli* in the benthic zone from 100 - 820 m downstream of the WWTP effluent during baseflow conditions in July 2017. The best-fit parameters from the model calibration were used to project the concentrations of *E. coli* for the duration of the sampling period (Fig. 1B) and estimate presence in surface water, benthic sediment, and the deeper bed. More information on the model parameters and range in values considered is discussed in Text S1.

3. Results

We first show the presence and absence of viruses and bacteria in the study (Section 3.1) and then the quantitative *E. coli* concentrations in the benthic sediment measured during the summer low baseflow conditions and simulated with the particle tracking model (Section 3.2). Finally, the calibrated parameters and in-stream velocity, used as a variable input parameter, were used in the particle tracking model to project longitudinal accumulation, transport and residence time of pathogens in benthic sediment at baseflow vs. stormflow conditions (Section 3.3).

3.1. Spatial variation in the presence or absence of bacteria and viruses in benthic sediment

Trace concentrations of viruses (NoV and RoV) were found in the benthic sediment during the summer baseflow conditions (Table 1). Virus presence varied longitudinally without any consistent spatial pattern. For instance, presence of NoV in benthic sediment in June was found in sampling locations nearer to the source and only at  $x = 300$  m in July. In comparison, there was an absence of RoV in June, but presence in 4 of the 5 sampling locations in July. In July, *E. coli* was present in the benthic sediment at all downstream sampling sites besides  $x = 623$  m.

Table 2

Best-fit model parameters for *E. coli* measured in the benthic sediment during baseflow conditions (Fig. 3).

Best-fit model parameters	
$c_D$ ( $s\ m^{-2}$ ), deposition coefficient	$5.5 \cdot 10^1 \pm 2.7 \cdot 10^1$
$c_R$ ( $s\ m^{-2}$ ), resuspension coefficient	$1.4 \cdot 10^{-1} \pm 8.0 \cdot 10^{-2}$
$p_R$ , probability of particle resuspending to the water column vs. being transported to the streambed. Being transported to the streambed has probability $1 - p_R$	$7.9 \cdot 10^{-2} \pm 1.0 \cdot 10^{-1}$
$\beta$ , Power-law exponent of the residence time distribution in streambed, controls particle release back to the benthic zone	$2.6 \cdot 10^{-1} \pm 6.6 \cdot 10^{-2}$

3.2. Model fit to benthic sediment *E. coli* measurements during low baseflow conditions

In July, *E. coli* concentrations in the benthic sediment peaked at  $x = 100$  m from the source ( $4.99 \cdot 10^2\ \text{gc}/\text{cm}^2$ ) and in general decreased with distance from the WWTP effluent (Fig. 2). The particle tracking mobile immobile model output matched the data observed during the July summer low flow sampling date (Fig. 2). Ranges in best fit model parameters are shown in Table 2 and the model error vs. parameter values are plotted in Fig. S2 to demonstrate parameter sensitivity of best-fit model parameters of observed *E. coli* concentrations in benthic sediment.

Although more data may be needed to validate the parameters for varying hydrologic conditions, the obtained parameters fall within the expected ranges shown in Drummond et al. (2022b) (Table S1). This match indicates that the obtained values were realistic and can be used as a basis to describe the role of benthic sediment during the low baseflow conditions during the study period and then use the model to project the spatial and temporal variability of *E. coli* presence in benthic sediment under dynamic flow conditions (Section 3.3). To assess how transport into and out of the benthic sediment could lead to the persistence of pathogens during low baseflow conditions, we calculated rates of exchange and residence times in the water column and benthic sediment (Table 3) using the best-fit parameters (Table 2). On the day of sampling, the average in-stream velocity was measured to be 0.051 m/s. There was only a small variation in daily velocity measurements in the month preceding the measurement (monthly velocity mean =  $0.055 \pm 0.003$ ), representing stable baseflow conditions. At the time of sampling, the exchange into the benthic sediment was calculated with the best-fit parameter for  $c_D$  and the in-stream velocity as  $\Lambda_D = 1.4 \cdot 10^{-1}\ 1/\text{s}$ , indicating an average in-stream water residence time prior to deposition (i.e.  $1/\Lambda_D$ ) of  $\sim 7$  s (Table 3). The majority of the bacteria was transported deeper into the streambed instead of resuspended back to the

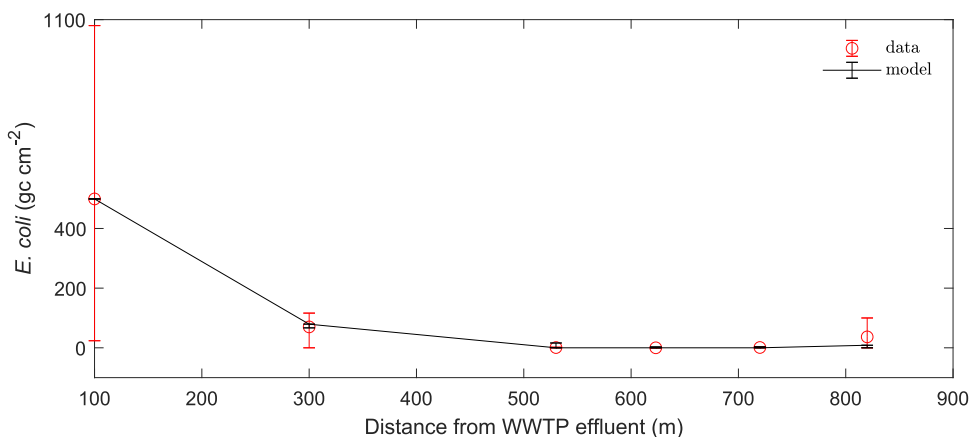


Fig. 2. Data vs model at sampling sites 100–820 m downstream of a WWTP effluent point source during baseflow conditions (July). Best-fit parameters shown in Table 2.

**Table 3**

Estimates of rates and residence times during baseflow and stormflow periods, identified in Fig. 1B. Baseflow velocity = 0.051 m/s and peak stormflow velocity = 1.78 m/s.

	Baseflow	Stormflow (peak)
$\Lambda_D$ ( $s^{-1}$ ), Exchange rate from the water column to benthic zone = $c_D v^2$	$1.4 \cdot 10^{-1}$	$1.7 \cdot 10^2$
$\Lambda_R$ ( $s^{-1}$ ), Resuspension rate from the benthic zone = $c_R v^2$	$3.6 \cdot 10^{-4}$	$4.4 \cdot 10^{-1}$
Water column residence time ( $1 / \Lambda_D$ , s)	$7.1 \cdot 10^1$	$5.9 \cdot 10^{-3}$
Benthic sediment residence time baseflow ( $1 / \Lambda_R$ , s)	$2.8 \cdot 10^3$	$2.3 \cdot 10^0$

water column, corresponding to a small resuspension probability ( $p_R = 7.9 \cdot 10^{-2}$ , Table 2). Exchange out of the benthic sediment, either deeper into the bed (92.1 %) or back to the water column (7.9 %), occurred at a rate of  $\Lambda_R = 3.6 \cdot 10^{-4}$  1/s, calculated with the average in-stream velocity and best-fit resuspension coefficient  $c_R$ . The resuspension rate indicated an average residence time in the benthic sediment of  $\sim 46$  min prior to resuspension (mainly to the deeper bed, but 7.9 % back to the water column) during baseflow.

The same calculations were made for the peak of the stormflow, identified in Fig. 1B, to compare to the baseflow estimates (Table 3). The rate of exchange into and resuspension out of the benthic zone during stormflow is three orders of magnitude higher than during baseflow (Table 3). In turn, the residence time in both the water column and benthic sediment was much lower during storm flow, 5.9 milliseconds and 2.3 s, respectively.

### 3.3. Model projections to compare longitudinal transport at baseflow vs. stormflow

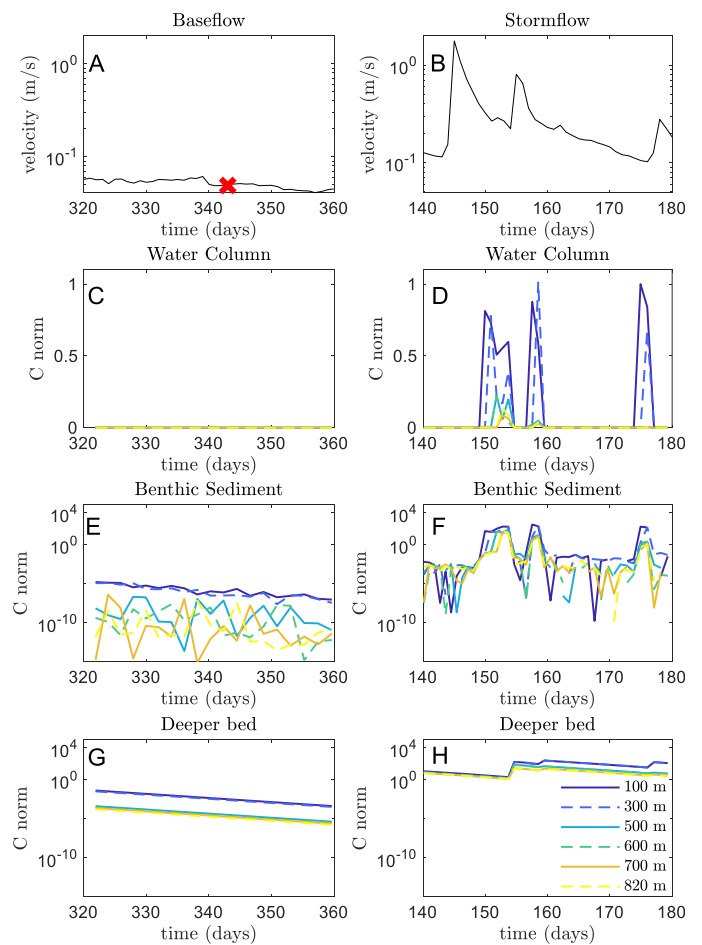
The best-fit model parameters were used to project *E. coli* concentrations in the water column (Fig. 3CD), benthic sediment (Fig. 3EF), and deeper bed (Fig. 3GH) over forty days during baseflow (LHS) and stormflow (RHS), encompassing a wide range of dynamic hydrologic conditions. The model output predicts varying concentrations of *E. coli* in the three zones that are dependent on the location ( $x = 100, 300, 530, 623, 720, 820$  m downstream of the wastewater treatment plant) and baseflow vs. stormflow hydrologic conditions.

## 4. Discussion

### 4.1. Controls on the spatial variation of pathogens in benthic sediment

Spatial patterns of virus accumulation, as observed for RoV and NoV in this study, are expected to differ based on pathogen type because of their differing transport properties, environmental conditions, attachment efficiencies, and inactivation rates (Bradford et al., 2013). Other studies also evidenced the presence of human enteric viruses in river sediment (Ali et al., 2004; García-Aljaro et al., 2017). With our inconsistent presence of NoV and RoV in benthic sediment with distance downstream from the source, it is also important to consider that the ability to observe the presence of pathogens is also dependent on the method choice. For instance, the efficiency of detection methods for viruses in complex samples such as biofilms and sediment are affected by several environmental and methodological limitations, especially if the samples are close to the lower limit of detection. Previous studies were also only able to qualitatively access enteric viruses in complex environmental samples (Hamza et al., 2009; Mackowiak et al., 2018). Despite these limitations, the current study shows that RNA of human enteric viruses can be detected in sediment using RT-qPCR, and infectious human enteric viruses might be present in benthic sediment and thus be a potential risk for public health.

Benthic sediment is expected to play a role in virus persistence because both solid and semisolid matrices in water bodies can be highly



**Fig. 3.** Velocity (A, B) and *E. coli* in the water column (C, D), benthic sediment (E, F), and deeper bed (G, H) during baseflow (LHS) and stormflow (RHS) conditions throughout the year as defined in Fig. 1B at  $x = 100, 300, 530, 623, 720, 820$  m downstream of the wastewater treatment plant effluent. Sampling date identified with a red x in A. C norm is the model output divided by the max water column value observed during stormflow (D) to provide a more direct comparison between the stream zones and flow conditions.

loaded with viruses of fecal origin and constitute potential repositories of both pathogenic human viruses and indicator coliphages (Hassard et al., 2016; Sidhu and Toze, 2009). Elmahdy et al. (2015) detected high concentrations of human adenovirus (HAdV) and RoV in sediment in a river in central Brazil. In fact, human adenovirus (HAdV) concentrations were 3 orders of magnitude higher in river sediment than in river water (Elmahdy et al., 2015). The exact role of sediment in the transmission of viral pathogens is still unknown and inactivation rates are yet to be determined for RoV and NoV (Martín-Díaz et al., 2020). Still, viruses deposited in sediment may pose an increased health risk as lower inactivation rates have been observed for viruses attached to sediment compared to viruses in the flowing water (Chung and Sobsey, 1993; Sakoda et al., 1997). One possible explanation for the lower inactivation rates in sediment, that can help explain their presence in the benthic sediment, is that adsorbed viruses have a smaller exposed surface for interaction with inactivating substances. Another reason is that viruses and bacteria are sheltered from solar radiation in benthic sediment (Bradford et al., 2013; Hassard et al., 2016). Further, viruses are not necessarily permanently adsorbed to sediment and may be mobilized by changes in water characteristics (pH, ionic strength and organic matter concentrations) (Chetochine et al., 2006; Wong et al., 2012), which combined with transport processes can lead to benthic sediment as a source of pathogens in-stream, explored further in Sections 4.2 and 4.3. In addition to transport and retention of viruses, other controls on

pathogenic virus persistence include sediment size and texture; the characteristics of the virus (mainly their size and isoelectric point); the pH, di- and trivalent cation concentration and dissolved organic matter in the flowing water; and temperature, moisture, aeration level and microbial activity in the sediment (Bradford et al., 2013; Fongaro et al., 2017; Hurst et al., 1980; Sinton et al., 1997; Sobsey et al., 1980). For example, finer streambed sediment will increase deposition rates due to enhanced filtration and trapping within narrow porewaters (Bradford et al., 2013). Consequently, the likelihood of pathogen resuspension back to the water column will decrease as there is a higher probability that pathogen detachment and flow through the porewaters will quickly lead to another deposition event. Therefore, with finer sediment, there is also increased dependence of pathogen resuspension on bed mobilizing flows, with flashy observations of increased resuspension during an event (e.g. Harvey et al. 2012). Finally, further studies that measure a variety of pathogens in benthic sediment are needed to differentially quantify their persistence, particularly as relationships among fecal indicator bacteria and waterborne pathogens are often not correlated (Bradshaw et al., 2016; Liao et al., 2015).

#### 4.2. Model parameters convey benthic sediment as a large store but small source of pathogens during baseflow conditions

By using the particle tracking model, that mechanistically describes how pathogens transport along the stream, we are able to improve our understanding on the rates of deposition to and resuspension from the benthic sediment that lead to pathogen accumulation in the benthic zone and deeper streambed. This study expands beyond the initial validation of the new particle tracking model (Drummond et al., 2022b) to evaluate the longitudinal transport and persistence of pathogens in a stream that receives continuous inputs from a wastewater treatment plant; and which is subjected to natural storm events. By combining the available empirical data on the longitudinal transport of *E. coli* and the long-term records of stream flow and precipitation data, we were able to make flow-dependent predictions of pathogen transport vs. persistence in the stream reach under a wide range of flow conditions (i.e., from typical baseflow to stormflow).

Benthic sediment acted as a store and only a small source of pathogens during baseflow conditions. More specifically, the deposition coefficient was two orders of magnitude higher than the resuspension coefficient (Table 2), matching previous observations of net deposition of microbes during baseflow conditions (Drummond et al., 2014b; 2015; 2022b). Net immobilization is expected based on the wide range of immobilization processes in the hyporheic zone (Boano et al., 2014). The deposition rate fell within the expected ranges for streams that have measured hyporheic exchange rates from the water column to the underlying sediment (Cheong et al., 2007; Drummond et al., 2020), and the value fell in between the deposition rates estimated using the same model framework in a different stream in Drummond et al., 2022b. Moreover, the average residence time of pathogens in the benthic sediment was only ~46 min prior to transport mainly into the deeper bed (based on the small resuspension probability  $p_R = 7.9 \cdot 10^{-2}$ , Table 2). The majority of the longer-term retention (~ months to years) takes places in the deeper bed, where microbes are slowly released back into the benthic sediment and eventually into the water column during baseflow. A low  $p_R$  aligns with previous model results (Drummond et al., 2022b) and other observations of pathogen transport during baseflow (e.g., Drummond et al., 2014ab). Furthermore, the average residence time fell within the estimated ranges of 31 min to 4.4 h using the same model framework (Drummond et al., 2022b), demonstrating that the majority of the long-term accumulation occurs in the deeper bed. Therefore, during baseflow conditions, the benthic sediment is a net store of pathogens with the majority of transport into the deeper bed and only a small source to the water column.

An interesting model result relates to the longitudinal variability in benthic sediment concentrations during baseflow. Concentrations in the

benthic sediment did not exhibit a simple relationship of decrease in concentration with distance from the source. Specifically, the model captured the high variability in *E. coli* benthic sediment concentrations (Fig. 3E). The high variability explains why we observed higher concentrations further downstream of the source on our sampling date during low baseflow conditions (i.e., higher concentrations at 820 m compared to 623 and 720 m (Fig. 2)). Benthic sediment concentrations are known to be highly variable in space and time, and in particular *E. coli*, has been shown to be based on local hydrologic conditions and sediment type (Stocker et al., 2018). Our model provides a mechanistic view that ties this complex variability to the competition between flow-dependent deposition and resuspension processes and distance from the source.

#### 4.3. Improved mechanistic understanding of benthic sediment as a pathogen store and source during stormflow

Our particle tracking model simulations aim to provide a mechanistic explanation for previous empirical results showing increased concentrations of fecal bacteria in streams and rivers after rainfall events (García-Aljaro et al., 2017; Madoux-Humery et al., 2016; Strathmann et al., 2016). Mackowiak et al. (2018) shows that concentrations of fecal indicator bacteria, such as *E. coli*, rose by approximately 2.5 orders of magnitude in the water column with the increase of rainfall and river flow rate. Although baseflow water column *E. coli* concentrations were negligible (Fig. 3A), resuspension of microbes into the water column leading to higher concentrations was observed during dynamic flow conditions (Fig. 3D). An important process incorporated into the model framework as opposed to previously available models, is the resuspension of pathogens during sub-critical flows, below a bed-mobilizing threshold that matches previous field results (Bradshaw et al., 2016; Fluke et al., 2019; Muirhead and Meenken, 2018; Park et al., 2017). Resuspension occurs because of turbulence and hyporheic flow processes that aid in the reversible filtration and resuspension of pathogens deposited in the benthic zone (Roche et al., 2019; Harvey et al., 2012). Only a portion of the deposited pathogens remobilize during the storm events, matching previous observations (Drummond et al., 2014; Stocker et al., 2018), to provide a more mechanistic representation to help us further explore the longitudinal impact of this phenomenon.

Even though net resuspension is observed during storm events, there is still increased exchange into the benthic sediment at this time, but with much shorter residence times (Table 3). In fact, during stormflow conditions with higher flows, there was increased exchange and lower residence times in the benthic sediment than during baseflow and increased longitudinal transport and connectivity from the source towards the end of the 820 m sampling reach. This increase in longitudinal connectivity is indicated by the similarity in *E. coli* concentrations observed in all sampling locations during stormflow conditions in both the benthic sediment (Fig. 3F) and deeper bed (Fig. 3H). However, at the end of the stormflow sampling period, the concentrations of *E. coli* concentrations started differing between close to the source (100–300 m) and the downstream sampling sites (530–820 m). This pattern can be explained by a decrease in water velocity at the end of the stormflow sampling period (Fig. 3B on and after 160 days), which likely resulted in a decrease in longitudinal connectivity. Overall, the *E. coli* concentrations in the deeper bed are more stable during both baseflow and stormflow as compared to the water column and benthic sediment, with increased concentrations found closer to the source (Fig. 3GH). Although the model predicted higher *E. coli* concentrations in the deeper bed compared to the benthic sediment, this number is distributed along the depth of the bed and therefore encompasses a much larger area than the benthic zone. This distribution in the deeper bed is not treated explicitly in our model, but microbial concentrations were previously shown to decrease with depth following a logarithmic profile (Drummond et al., 2014b). Although the benthic sediment is overall a source of pathogens to the water column during storm flow, any accumulation in



the deeper bed is still disconnected from the water column during a typical stormflow. Therefore, the deeper bed is still a store of these pathogens that can be slowly released back to the benthic sediment, as represented by the particle tracking model that incorporates the exchange between these zones as dependent on changing velocity. Model results provided a mechanistic understanding of how pathogens are transported longitudinally and between water and transient storage zones in response to baseflow and stormflow transport dynamics that determine spatial and temporal accumulation patterns in the streambed.

## 5. Conclusions

Through particle tracking modeling supported by empirical data of pathogen presence in benthic sediment, we evaluated the role of benthic sediment as a store and source of pathogens in an intermittent stream during dynamic flow conditions. Our combined measurement and modeling approach can help bridge gaps in data, as it is still unfeasible to experimentally monitor the high spatiotemporal resolution often needed in microbial studies, and available observations are still limited. These results provided insight to longitudinal accumulation, transport and residence times that control microbial risk in streams. This study demonstrated:

- Accumulation patterns of pathogens in benthic sediment are expected to vary spatially and temporally.
- A particle tracking mobile immobile model with water column, benthic sediment, and deeper bed compartments can match the spatial variation of pathogens in benthic sediment during low baseflow conditions.
- During stormflow, rates of exchange increased and residence times in benthic sediment decreased, leading to increased longitudinal connectivity and similar *E.coli* concentrations from up to downstream of the pathogens source.
- Continuous transport into and out of the benthic sediment regulates the presence of pathogens and viruses in the stream water column as can be represented by a particle tracking model that simulates dynamic flow conditions.

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Jennifer D Drummond reports financial support was provided by National Science Foundation. Jennifer D Drummond reports financial support was provided by European Research Council. Tomas A Aquino reports financial support was provided by European Research Council. Eugenia Marti, Susana Bernal, Esperanca Gacia reports financial support was provided by Spanish Scientific Research Council. Tomas A Aquino reports financial support was provided by French National Research Agency.

## Data availability

The data is included in the main text and the source code for the model is publicly available online.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.watres.2023.120637](https://doi.org/10.1016/j.watres.2023.120637).

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