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Evaluating Bacteriophage P22 as a Tracer in a Complex Surface Water System: The Grand River, Michigan

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Viruses are important pathogens in both marine and fresh water environments. There is a strong interest in using bacteriophages as tracers because of their role as model viruses, since dissolved chemical tracers may not adequately describe the behavior of viruses that are suspended colloids. Despite a large number of studies that examined the transport of bacteriophages in the subsurface environment, few studies examined phage transport in large and complex surface water systems. In this paper we report the results of a dual tracer study on a 40 km reach of the Grand River, the longest river in Michigan, and we examine the performance of bacteriophage P22 relative to a chemical tracer (Rhodamine WT). Our analysis based on the transient storage (TS) model indicated that P22 can be successfully used as a tracer in complex surface water environments. Estimated P22 inactivation rates were found to be in the range 0.27−0.57 per day (0.12−0.25 $\log_{10}$ per day). The highest inactivation rate was found in a reach with high suspended solids concentration, relatively low dissolved organic carbon content, and sediment with high clay content. Estimated TS model parameters for both tracers were found to be consistent with surficial geology and land use patterns. Maximum storage zone sizes for the two tracers were found in different river reaches, indicating that different processes contributed to TS within the same reach for the two tracers. This model can be used to examine the arrival times and concentrations of human viral pathogens released from untreated sewage at recreational areas.

Introduction

Bacteriophages are increasingly being used as tracers in hydrology because of their low detection limit, rapid and inexpensive enumeration methods (*1*), and their role as model viruses (*2, 3*). Bacteriophages are viruses that selectively invade specific bacterial cells and have no adverse effect on humans or animals. Viruses were implicated in 80% of the disease outbreaks for which etiological agents were identified in the past (*4*). Abundance tests (e.g., ref *5*) have shown that viruses are ubiquitous in both the water column and sediments of marine and fresh waters. Of the major rivers sampled in Michigan, 33% tested positive for the presence of viable enteric viruses, and they are suspected to be the chief cause of swimming-associated diseases in recreational waters (*6*). Specifically, point sources of inadequately treated sewage discharge from urban areas can be one of the most significant virus pollution contributors (*7*). The majority of studies investigating virus fate have been performed under laboratory conditions (*8*) or in the subsurface environment (e.g., ref *9*). In natural streams, statistical approaches were used in the past (e.g., refs *10* and *11*). Earlier studies mostly focused on virus removal in constructed wetlands (*12–14*) and waste stabilization ponds (*2*). In a study conducted on the Areuse River in Switzerland (*1*), bacteriophage H40/1 was used with the chemical tracer uranine. The authors reported similar distribution patterns for the two tracers but a 20% higher recovery rate for the phage compared to uranine. To the best of our knowledge, the performance of bacteriophages as surface water tracers (e.g., slug injection experiments) has not been evaluated in major rivers in the past. In the few studies reported in the literature, the focus was mainly on travel times and recovery rates, and the role of environmental factors contributing to phage inactivation/attenuation has not been explored.

Bacteriophages are known to undergo natural decay or inactivation as time progresses, adsorb readily to suspended particulate matter, and due to their colloidal nature, can aggregate into clumps large enough to settle out of the water. Factors that influence the inactivation of bacteriophages in the natural environment are complex and varied and have been described in several excellent review articles (*8, 15, 16*). Temperature and solar radiation are generally regarded as important contributing factors in surface waters (*8*). P22 is an icosahedral-shaped DNA bacteriophage, 52−60 nm in size, that belongs to the family podoviridae, contains dsDNA that is approximately 43 400 bp, and has a very short tail (*17*). Bacteriophage P22 infects smooth strains of *Salmonella typhimurium* (those that carry O-antigen surface polysaccharide). It was confused with PRD1 at one time, used extensively in groundwater tracer studies, and has recently been genetically characterized by our laboratory. The current strain used in our laboratory was shown to have inactivation rates of 0.02−0.05 $\log_{10}$ per day in groundwater at temperatures below 25 °C (*15*).

Transport of solutes in rivers is controlled by dispersion as well as by transient storage (TS), which includes contributions from both surface storage (e.g., due to in-stream vegetation) and hyporheic exchange (due to interaction with near-bed sediments). TS can significantly delay the downstream transport of solute mass. In large streams, the transport of solutes is also influenced by catchment hydrology and watershed characteristics. Using Rhodamine WT (RWT) as a tracer, Gooseff at al. (*18*) recently found that increased geomorphic complexity from urban to natural settings in a Wyoming stream increased the potential for TS. Few studies, however, examined the impact of watershed-scale processes on TS in mixed land-use settings. In addition, the differences between responses of chemical and biological agents in these settings remain largely unknown.

The aims of this paper were to (1) evaluate the performance of bacteriophage P22 in a major US river (the Grand River, Michigan) relative to RWT, (2) to evaluate the relative importance of different environmental factors that influence the inactivation (loss of virus per unit time) of bacteriophage

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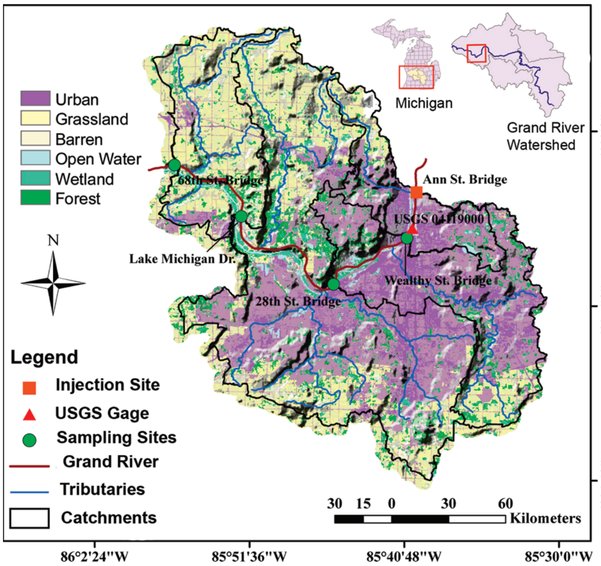

FIGURE 1. Map of the Grand River watershed and the study region showing the sampling sites.

P22, and (3) to examine the behavior of both tracers as related to catchment-scale properties and processes.

Site Description

The tracer study was conducted on a 40 km stretch of the Grand River, a 420 km long tributary to Lake Michigan (Figure 1), starting from the city of Grand Rapids and extending to Coopersville. Surficial geology of the Grand River Basin is dominated by rivers crisscrossing the moraines and outwash plains formed by extensive glaciation during the Pleistocene (Supporting Information). Till plains, moraines, kames, and eskers of the Port Huron system are the predominant surface features. The Ann Street Bridge near downtown Grand Rapids was selected as the injection point. This location is close to the combined sewage overflow (CSOs) outfalls that serve the city of Grand Rapids. These CSOs are point sources of pathogens discharging into the Grand River. One of the objectives of the current study was to examine the potential health risks posed by the CSO discharges. Sampling was carried out at four downstream sites; bridges (Wealthy Street, site 1; 28th Street, site 2; Lake Michigan Drive, site 3; 68th Street, site 4) and distances from the injection site are given in Table S1 (Supporting Information). The study reach was sufficiently long to make watershed influences important. A U.S. Geological Survey (USGS) streamflow gaging station (04119000) is located 1.05 km upstream from the first sampling site. The discharge on the test date was on the end of a recession limb (Figure S1 in the Supporting Information) with the values during the experiment gradually declining from 3230 to 3010 cubic feet per second. The study reach is a perennial gaining stream. No precipitation event was reported in the three days prior to the experiment. Contributions from the tributaries and baseflow together, termed lateral inflow, are important to correctly describe the downstream transport of both tracers. Although lateral inflow is often neglected in tracer studies conducted on relatively short river reaches, it is not negligible in our case.

Materials and Methods

Rodamine WT 20% (weight) solution was used in the study. P22 was obtained from Samuel Farrah, University of Florida, Gainsville, Florida and was maintained on the host *Salmonella typhimurium* LT-2 (ATCC 19585). P22 stock was grown by inoculating 100 ml of log-phase *S. typhimurium* host with 1 mL of P22 stock ($\sim 10^{11}$ pfu/mL) and incubated at 37 °C for approximately 3–5 h. After incubation, 0.01 g of lysozyme and 3 mL of 0.2 M sterile EDTA were added to the flask and mixed well. The culture was then centrifuged at 4000 rpm for 10–15 min, and the supernatant was filter sterilized through a 0.45 μm membrane. P22 stock was stored at 4 °C until used.

RWT and P22 solutions were injected into the river (slug release) from the Ann Street Bridge on May 8, 2006 at 7:00 a.m. A total of 8770 g of RWT and 16 L of bacteriophage P22 (4×10^{11} PFU/ml) were released. At each station, grab samples were collected from just below the surface using manual sampling. Two samples were taken at the same time. One was stored in a dark cooler for RWT analysis. A 5× trypticase soy broth (TSB) was added to the other sample to stabilize the bacteriophage for P22 analysis. All samples were kept on ice and were analyzed within 48 h in the laboratory. Meanwhile, water temperature, pH, suspended solids, and weather data (i.e., ambient temperature, rainfall, wind, etc.) were noted during sampling. A Turner Designs 10 AU field fluorometer (Turner Designs, Inc., Sunnyvale, California) was used to initially detect the dye at the first three sites. The sampling frequency for both tracers was increased after receiving a RWT signal. RWT samples were analyzed in the laboratory using the same 10 AU unit. Water samples were assayed for P22 following the double agar layer procedure (*19*). Samples from site 1 were diluted to a 10^{-3} concentration, and between 1 and 2 mL of each sample in at least duplicate were assayed for the phage presence on tryptic soy agar. The plates were incubated for 24 h at 37 °C. The detection limit of this method is less than one plaque-forming unit per milliliter. Total suspended solids concentration was determined according to Standard Method 2540-D—total suspended solids dried at 103–105 °C (*20*).

The river is significantly wider in comparison with the numbers reported in many previous tracer studies. Therefore, to obtain a better idea about the lateral variability at each station, sampling was done at multiple locations (left, right, and center) on each bridge except at site 3, where sampling was done at two locations (left and right) due to the presence of an island in the middle of the channel. Because of the long travel time to site 4, P22 data was not collected. In addition to the manual sampling, a submersible fluorometer (Turner Designs SCUFA) equipped with a data logger and programmed to measure RWT concentrations every 10 s, was deployed at sampling sites 1, 3, and 4. Discharge at all the sampling locations was measured using a Teledyne - RD Instruments (1200 kHz) Rio Grande acoustic doppler current profiler (ADCP).

Analysis. Solute transport in the longitudinal direction was described using the TS formulation (*21*). The ability of the TS model to describe solute transport in natural streams is well documented (*22*). Briefly, the model solves two separate equations — one for solute concentration in the main channel and another in the storage zones. Solute exchange between the bulk flow and the storage zones was described using a first-order exchange coefficient, α. The governing equations appear as shown below (*21*).

$$\frac{\partial C}{\partial t} = -\frac{Q}{A}\frac{\partial C}{\partial x} + \frac{1}{A}\frac{\partial}{\partial x}\left(AD\frac{\partial C}{\partial x}\right) + \frac{q_L}{A}(C_L - C) + \alpha(C_s - C) - kC \tag{1}$$

$$\frac{\partial C_s}{\partial t} = \alpha\frac{A}{A_S}(C - C_s) - kC_s \tag{2}$$

Here, Q denotes the discharge, A and A_S are the cross-sectional areas of the main channel and the storage zones, respectively, C and C_S are the concentrations in the main channel and the storage zones respectively, D is the dispersion coefficient, q_L is the lateral inflow, α is the mass exchange coefficient between the main channel and the storage zones, and k is

TABLE 1. Estimated Parameter Values for the TS Model for RWT and P22

reach	A (m^2)	A_s (m^2)	D (m^2/s)	$\alpha \times 10^{-5}$ (s^{-1})	q_L (m^3/s/km)	k_0 (d^{-1})	k_I (d^{-1}/kW)	A_s/A	t_S (hours)	F_{med}^{200} %	average velocity (m/s)
1 (RWT)	223.2	5.00	2.16	2.82	0	0	0	0.022	0.22	0.03	0.41
2 (RWT)	174.5	24.53	1.60	7.98	0.132	0	0	0.140	0.49	0.40	0.52
3 (RWT)	171.3	22.74	4.20	5.75	0.219	0	0	0.133	0.64	0.27	0.53
4 (RWT)	172.7	17.81	1.39	5.90	1.040	0	0	0.103	0.49	0.22	0.44
1 (P22)	216.3	9.94	1.34	8.75	0	0	0	0.046	0.15	0.19	0.42
2 (P22)	178.0	19.88	3.19	6.05	0.132	2.03×10^{-3}	0.38	0.112	0.51	0.25	0.51
3 (P22)	167.2	28.55	1.87	7.80	0.219	0.46	0.47	0.171	0.61	0.44	0.55

the first-order inactivation/decay rate (for P22). C_L is the concentration associated with lateral inflow (assumed zero in our case). To obtain parameters that are representative of the conditions at each site (bridge), we used the flow-weighted concentrations of our observed data (based on our manual sampling) for the TS modeling. The flow-weighted concentrations for each site were computed using the discharge data as shown below,

$$\bar{C}(x, t) = \frac{q_L C_L + q_C C_C + q_R C_R}{q_L + q_C + q_R} \tag{3}$$

where q_L, q_C, and q_R are fractional discharges ($Q = q_L + q_C + q_R$) estimated from ADCP measurements and C_L, C_C, and C_R are the observed concentrations (either RWT or P22). Equations 1 and 2 were applied on a reach basis, and parameters were estimated separately for RWT and P22. The model was numerically solved using a fourth-order accurate compact scheme (*23*). The parameters of the model, A, A_S, D, α, and q_L were estimated by minimizing the root mean squared error (RMSE) between the observed and simulated concentrations using a global optimization algorithm, the shuffled complex evolution (SCE-UA) (*24*).

Landuse/landcover data for the study region was obtained from Michigan Geography Data Library in the form of a thematic raster data set. This 30 m resolution data was derived from the classification of Landsat TM images collected from 1997 to 2001. The catchment of the four reaches was delineated from the digital elevation model (DEM) and digital hydrography data using the U.S. EPA BASINS program. The percentage of each landuse type was calculated in ArcGIS. In a gaining stream reach such as the present one, downstream solute concentrations are diluted by tributaries and baseflow contributions from groundwater entering into the stream. Quantification of dilution is important for describing P22 transport because estimates of inactivation must correctly separate the effects of dilution. RWT breakthrough data was used to estimate the dilution by estimating the parameter q_L in the TS model; however, to provide an independent check on the estimated values of q_L in different stream reaches, we used the watershed-scale hydrologic model based on SWAT (*25*). The SWAT model is a semidistributed model that describes the entire hydrologic cycle. A daily time step SWAT model for the Grand River watershed was constructed and calibrated against data from twelve USGS gages within the watershed. The tributary and basin output on the test date was extracted and was used to calculate lateral inflow for each reach. More information about the calibration and predictions of the SWAT model is available in the Supporting Information.

Results and Discussion

To evaluate the relative performance of the two tracers, we first estimated the fractional recovery of tracer mass by integrating the tracer breakthrough data assuming complete mixing:

$$f = \frac{1}{M_0}\int_0^\infty C(x, t)\,Q(x, t)\,\mathrm{d}t \tag{4}$$

Here, f is the fractional recovery (expressed as percent), $C(x,t)$ is the tracer concentration, $Q(x,t)$ is the discharge, and M_0 is the mass released. Because the actual discharge was not available at all spatial locations (x), it is difficult to accurately estimate the recovery. Recovery of RWT and P22 estimated using the discharge from the USGS gaging station is summarized in the Supporting Information. RWT recovery at sites 1 and 2 was greater than 100%. This is attributed to a combination of factors, including experimental error, discharge measurement error, and errors in the initial mass released (M_0). Recovery values between 115% and 85% have previously been reported in the literature (*26*). RWT recovery at site 3 was underestimated because the breakthrough was not complete. For P22, however, the recovery at the first site was only 31%, although it remained fairly constant at the other sites (note that breakthrough was incomplete at site 3). The low recovery of P22 is attributed to the turbulence generated by a weir in downtown Grand Rapids (in reach 1) as well as to potential losses associated with aerosolization of P22 (while releasing from the bridge), resulting in overestimation of the initial mass M_0. Such low recovery has been reported in the literature previously (*12*). Due to uncertainty in the initial mass released and to facilitate comparisons with mathematical models, the mass estimated at site 1 was used as the initial mass in our TS modeling for both RWT and P22 (*26*). Computed first moments (mean arrival times) were almost identical for RWT and P22 (Supporting Information).

To estimate the inactivation rate of P22, the dilution effect from lateral inflow must be correctly estimated. Estimates of lateral inflow from the watershed model and independently from the TS modeling were found to be very similar, suggesting that both approaches are valid. Loss/inactivation of P22 includes contributions due to solar radiation as well as other environmental factors such as temperature, pH, virus sorption to river bed material and suspended particles etc. Our inactivation formulation separates these effects using eq 5,

$$k = k_0 + k_I I(t) \tag{5}$$

where k_I is the rate of inactivation due to solar radiation (d^{-1} kW^{-1}), $I(t)$ is the net shortwave radiation (kW) as a function of time (and geographical location) estimated using an algorithm proposed in ref *28*, and k_0 denotes the loss due to all other factors, including the effects of temperature and sedimentation. Estimated parameters of the TS model based on eq 5 are presented in Table 1. Comparisons between the observed and simulated concentrations are given in Figures 2 and 3. Overall, the TS model was able to adequately describe the behavior of both tracers. The average first-order inactivation rate (k) estimated using eq 5 in reaches 2 and 3 was found to be 0.27 and 0.57 per day (0.12 and 0.25 $\log_{10}$ per day), respectively. These rates represent net losses of P22 in the water column and are similar to the rates reported in the literature for surface waters. For example, a value of 0.3 per day was reported in ref *13* for bacteriophage PRD1 in a constructed wetland with significant surface flow. We notice that similar values of k_I were obtained for both reaches,

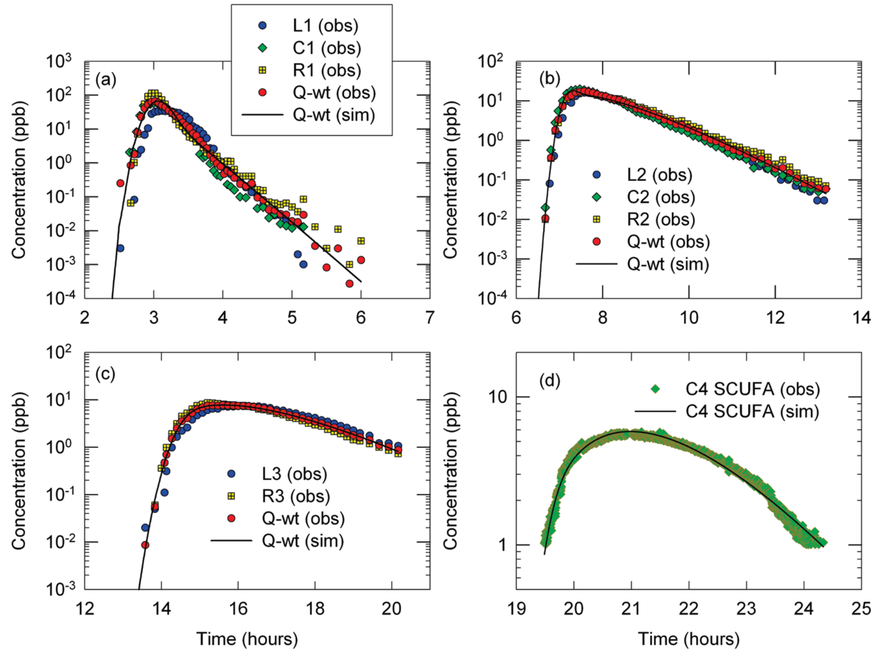

FIGURE 2. Comparisons between observed and simulated tracer concentrations for Rodamine WT. Rhodamine WT breakthrough curves at the four sampling locations (bridges 1−4). L, C, and R denote the left, center and right sampling locations, respectively. "Q-WT" denotes the flow-weighted concentrations for each bridge. Symbols denote the observations, and the solid lines denote simulations.

suggesting comparable solar inactivation effects in the two reaches. However, the k_0 value changed dramatically between the two reaches. Water temperature increased by only about 2 °C between reaches 2 and 3, and pH increased from 8.5 to 9.0; therefore, effects of temperature are expected to be relatively small (*16*). Because solar radiation was already accounted for in k_I, the difference in k_0 can only be attributed to other factors such as the organic content of water, inorganic suspended matter, the presence of salts, etc. Total suspended solids (TSS) concentration, dissolved organic carbon (DOC), percent silt and clay content in the sediment, major ions, and conductivity as a function of distance are provided in the Supporting Information. Generally, total suspended solids and the percent clay content increased in the downstream direction, and this is consistent with the shift in land use from urban to agriculture. Multivalent cations (Ca^{2+} and Mg^{2+}) are known to form salt bridges between virus and solid surfaces, and their presence was indicative of high attachment rates. Viral association with suspended clay solids was found to be more efficient in the past. Humic substances in the organic matter are known to compete with viruses for the same binding sites. Therefore, a decrease in DOC in reach 3 could potentially result in higher attachment rates (*28*). ADCP transect data showed that reach 3 also had the lowest cross-sectional average velocity (compared to other reaches) due to a relatively deeper channel (Supporting Information), which indicates faster sinking fractions of particulate matter in the decelerating flow. Previous studies that examined the effects of sedimentation of different microorganisms in rivers are relatively few. Duran et al. (*29*) examined the removal and inactivation of bacteriophages in fresh water systems and concluded that the effect of sedimentation on removal was similar for all the microorganisms studied (i.e., both bacteria and bacteriophages). This information, coupled with the fact that reach 3 had the highest TSS, higher percentage clay, lower DOC, and the lowest cross-sectional average velocities, suggests that sedimentation was an important P22 removal mechanism in reach 3. In addition to these factors, viral aggregation may also potentially contribute to decreasing numbers in reach 3.

TS significantly modifies the distribution of the tracer mass after the initial mixing period (*26*). We note that both tracers have similar ranges of TS parameter values. For example, the TS zone residence time (*22*), defined as $t_S = (A_S/A)/\alpha$, increased in the downstream direction for both tracers. This is consistent with the surficial geology of the Grand River Basin. Vegetation in the riparian buffer zones, as well as the thickness of coarse-grained sediments, generally increased in the downstream direction. Therefore, we expect the relative importance of TS to increase in the downstream direction. Differences in the behavior of the two tracers are noted when we examined the metric F^{200}_{med} (Table 1), defined by Runkel (*22*) as the fraction of the median reach travel time that is due to TS for a standard reach length of 200 m. This parameter was defined as:

$$F_{\mathrm{med}} = [1 - \mathrm{e}^{-L\alpha A/Q}]\frac{A}{A + A_S} \tag{6}$$

where $L = 200$ in eq 6 for F^{200}_{med}. Larger F^{200}_{med} values indicate more prominent TS. For P22, F^{200}_{med} values increased in the downstream direction, and the highest values were found in reach 3. This is consistent with the geology and the fact that reach 3 had the highest channel sinuosity (defined as the ratio of the downstream length of the channel to the straight-line distance measured between the two end points (*18*)). A body of literature shows that river reaches with more complex geomorphic structure (larger sinuosity) have a higher potential for hyporheic exchange (*18*). The reach was also marked by the presence of large islands in the middle of the channel over part of its length (vegetation growing on the islands reached out into the channel during this study). All of these factors are expected to enhance TS and to delay the downstream transport of P22. However, RWT showed a slightly different trend. F^{200}_{med} values for RWT were found to be highest in reach 2. The high F^{200}_{med} value was obtained because the TS model predicted a higher value for the storage zone sizes (A_S) in reach 2. This result for RWT was somewhat unexpected given the fact that urban stream reaches are known to have very little TS (*18*). However, closer examination showed that reach 2 had the highest percent of wetlands

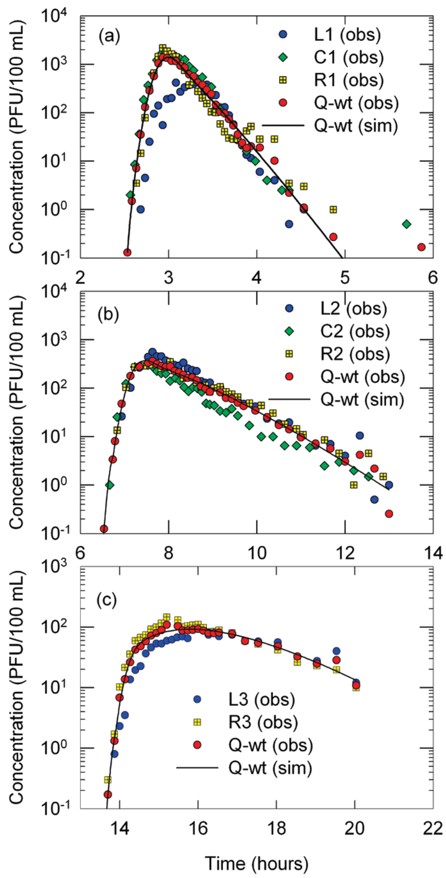

FIGURE 3. Comparisons between observed and simulated tracer concentrations for P22. Bacteriophage P22 breakthrough curves at the three sampling locations (bridges 1−3). L, C, and R denote the left, center and right sampling locations, respectively. "Q-WT" denotes the flow-weighted concentrations for each bridge. Symbols denote the observations, and the solid lines denote simulations.

flanking the river (Supporting Information). Earlier studies (*30*) showed that the high organic content in wetland environments could significantly enhance RWT adsorption to streambed materials and to plant detritus (*31*). Adsorption may have contributed to an enhanced storage zone size in reach 2 for RWT. As mentioned earlier, the high organic content in reach 2 could potentially decrease virus adsorption while increasing RWT sorption. Our results (particularly the F^{200}_{med} values), therefore, highlight the differences in the behavior of the chemical and biological tracers in different stream reaches in a complex surface water system.

Relating TS to land use characteristics and channel complexity is an active area of research (*18*), and few studies examined the differences in the behavior of chemical and biological tracers. In our first reach (Figure 1), the land use was predominately urban (52%). This reach, which was characterized by a wide, straight channel and silt loam streambed with little vegetation, had the smallest storage zone size as well as the smallest t_S value (residence time). This finding is in agreement with the results of Gooseff et al. (*18*), who found that urban streams have the least complexity and the least potential for TS. The catchment of the second reach had a similar land use composition with only 5% decrease in urban, 2% increase in agricultural, and 4% increase in forested land use; however, the estimated storage zone sizes and flushing times increased significantly (Table 1). Agricultural land use was the dominant land use type (45%) in the third reach, and urban land percentage decreased to 24%. This dramatic change in land use resulted in a significant increase in the TS for P22, but TS decreased slightly for RWT as explained earlier. Examination of the estimated TS zone sizes for RWT and P22 (Supporting Information) indicated that storage zone sizes correlated negatively with urban land use and positively with agricultural and forested land uses for both tracers.

The results of our dual tracer study indicate that bacteriophage P22 was a suitable tracer in a complex fresh water system as indicated by the travel times, reach-averaged velocities, percent mass recovery, residence times, moments, and storage zone characteristics. Stream chemistry and sediment characteristics influenced by the shifts in land use were found to be important for understanding the observed behavior of bacteriophage in different reaches. Differences in the behavior of the two tracers were highlighted by the F^{200}_{med} metric, which indicated that the two tracers had their peak storage in different reaches. The estimated inactivation rate (0.19 $\log_{10}$ per day, averaged over reaches 2 and 3) can be used to assist in predictive modeling efforts in the future to address public health warnings at recreational areas.

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Supporting Information Available

Additional details referenced within the text are provided as Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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