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Characterizing Water Circulation and Contaminant Transport in Lake Geneva Using Bacteriophage Tracer Experiments and Limnological Methods

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Multi-tracer tests with three types of marine bacteriophages (H4/4, H6/1, and H40/1), together with various limnological methods, including physicochemical depth profiling, surface drifters, deep current measurements, and fecal indicator bacteria analyses, have been applied to characterize water circulation and pathogen transport in the Bay of Vidy (Lake Geneva, Switzerland). The experimental program was carried out twice, first in November 2005, when the lake was stratified, and a second time during holomixis in February 2006. The bacteriophages were injected at three points at different depths, where contaminated waters enter the lake, including the outlet pipe of a wastewater treatment plant, a river, and a stormwater outlet. Thereafter, water samples were collected in the lake at 2 m depth during a 48 h sampling campaign. The results demonstrate that (i) contaminated river water spreads rapidly in the bay; (ii) a well-developed thermocline is highly effective in preventing contamination from the depth to rise up to the surface; (iii) rapid vertical mixing and pathogen transport occur under thermally homogeneous conditions; and (iv) repeated multi-tracer tests with bacteriophages are a powerful technique to assess water circulation and contaminant transport in lakes where high dilution occurs.

Introduction

Lakes are important drinking water resources in many parts of the world, although only 0.3% of the global freshwater stored in lakes and rivers (*1*). At the same time, untreated or only partly purified wastewaters from households, agriculture, and industry are frequently directly or indirectly released into lakes, so that there is a potential short circuit between wastewater and drinking water. This is particularly problematic for microbial pathogens (bacteria, protozoans, and viruses), which can be present in extremely high concentrations in wastewaters (often $>10^8$/100 mL), while already very low levels (sometimes <1/100 mL) may cause disease outbreaks (*2*, *3*). Therefore, even high dilution, as it occurs in large lakes, does not always solve the problem. Furthermore, some pathogens are highly resistant both in the environment and in drinking water treatment systems. There are three principal strategies as to how this problem can be addressed: better wastewater purification systems, better drinking water treatment systems, and a detailed knowledge of the water circulation, contaminant transport, and attenuation processes in lakes, which makes it possible to avoid or minimize the previously mentioned short circuit (i.e., to pump the water at locations and depths where it is not threatened by wastewater).

Lake Geneva (Lac Léman) is the most important freshwater reservoir of Western Europe with a volume of 81 km^3 and a maximum depth of 309 m (*4*). Approximately 700 000 people are supplied by water from this lake, and the water quality is under strict legal control. Also, the city of Lausanne, with 127 000 inhabitants (the largest city in the region), receives 58% of its freshwater from the lake. The water is pumped from a depth of 45 m at an average rate of 385 L/s. The most contaminated part of the lake is the Bay of Vidy, only about 3−4 km east of the drinking water pumping station. Previous studies mainly focused on heavy metals in the sediments (*5*, *6*), but there are also significant microbial contamination problems in the bay and on its tourist beaches, which are suspected to originate from different sources but have not been studied in detail yet.

To better understand water circulation and the propagation of bacteria and other contaminants in the bay, a comprehensive experimental program was carried out. It included the measurement of depth profiles of physicochemical parameters, fecal indicator bacteria (FIB), the use of drifters to investigate the near-surface currents, acoustic Doppler current profilers (ADCP) to measure deep currents, and multi-tracer experiments with three types of bacteriophages (H4/4, H6/1, and H40/1). The complete experimental program with bacteriophage tracing was performed twice: first in November 2005, when the lake was stratified, and a second time in February 2006 during holomixis. Additional depth profiling, drifter measurements, and bacteriological sampling were also performed at eight other times between March 2005 and February 2006.

Bacteriophages (short: phages) are viruses that infect specific host bacteria (i.e., each type of phage attacks one type of bacteria (*7*)). Therefore, selected marine phages can be used as artificial tracers in freshwater environments, where their natural background level is zero. These phages can be easily produced in large quantities (e.g., 10^{15} phages in 10 L) but detected at extremely low levels ($\sim$1 phage in 2 mL); furthermore, they are not harmful to humans and the aquatic environment. Phages are particularly useful to simulate transport and attenuation processes in saturated and un-saturated porous media, either by means of column or batch experiments in the laboratory (*8*, *9*) or field experiments in sand and gravel aquifers (*10*−*14*), flowing surface waters (*12*), waste stabilization ponds (*15*), or constructed wetland areas (*16*). Naturally occurring phages that infect fecal or patho-genic bacteria are increasingly used as contamination indicators (*17*). However, this study represents the first example of a multi-tracer test with bacteriophages in a lake.

Experimental Procedures

Study Area. The Bay of Vidy is located near the Lausanne city center on the northern shore of Lake Geneva (Figure 1). There are three main inflows of contaminated water into the bay: the wastewater treatment plant (WWTP), the Cham-

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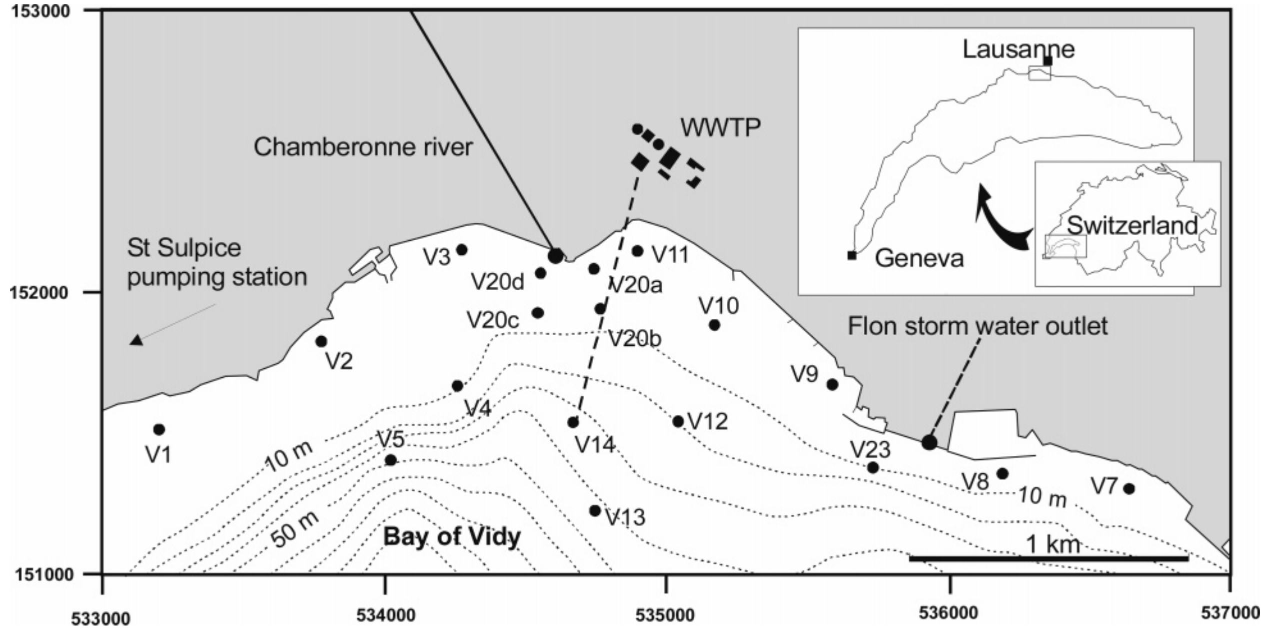

FIGURE 1. Map of the Bay of Vidy with the positions of the three point sources of contaminated waters (WWTP, Chamberonne, and Flon) and the sampling sites (Swiss coordinates, 1 km grid). The drinking water pumping station is located 3.8 km west of the WWTP outlet (V14).

beronne River, and the Flon stormwater outlet. The WWTP treats approximately 1−3 m^3/s urban wastewater; the purified water is discharged into the bay 700 m from the shore at 30 m depth. The Chamberonne River includes water from its natural drainage basin but also some untreated wastewaters. The Flon collects surface and wastewater from the western part of the city, which is usually treated in the WWTP but released into the lake during flood events via a conduit at 10 m depth. Figure 1 also shows the location of the sampling and measurement points for this study (V1−V23).

Limnological Parameters. Up to 40 m deep vertical profiles of temperature (*T*), electrical conductivity (EC), and other parameters (not discussed here) were measured using an YSI 600 XL multi-parameter probe. The measurements were performed at different locations in the bay (mostly V12, V14, and V14a−d) and repeated 11 times between March 2005 and February 2006 to assess the thermal and physicochemical state of the lake during an annual cycle and in particular during the two tracer tests. Points V14a−d are located about 150 m north, east, south, and west of V14, respectively.

Near-surface currents in the bay were measured during the two tracer tests (and at eight other times between March 2005 and February 2006) using drifters that sample a depth of 2 m and are thus not directly influenced by wind drag. They were released at different points and collected again after 5−8 h. GPS coordinates of initial and final positions were used to calculate their displacement vectors. Close to the WWTP outlet pipe, an ADCP flow meter was moored, which monitored continuously the speed and direction of water currents in vertical steps of 2 m between the bottom and the lake surface.

Production of Bacteriophages. The three phage types H4/4, H6/1, and H40/1 have been selected for this experiment because they proved to be suitable tracers for groundwater and flowing surface waters (*12*). The bacterial host of H40/1 has been identified as *Pseudolateromonas gracilis* (*7*), but the bacterial strains H4 for phage H4/4 and H6 for phage H6/1 have not been characterized yet. The phages were produced in the laboratory of LAMUN using a two-step procedure: (i) preparation of a highly concentrated culture of the respective host bacteria in marine peptone−yeast (PY) extract broth and (ii) addition of a small quantity of phages, which multiply by infecting and killing the bacteria (*18*). The PY broth contained the following constituents (per L): 5 g of peptone from casein (Merck, no. 1.07213), 1 g of yeast extract (Biolife, no. 4122202), and 24 g of sea salt (Instant Ocean, Aquarium Systems) and was adjusted to pH 7.2 before autoclaving. The produced phages (10 L) were stored at 4 °C in the dark until they were used within 1 week. However, according to our experience, such highly concentrated stocks ($\sim 10^{10}$ PFU/mL) can be kept under these conditions for a few months with only a minor decrease in the phage titer.

Injection of Bacteriophages. The first multi-tracer test was carried out on November 7, 2005 and the second one on February 21, 2006. For both experiments, the three types of phages were injected at the three sites where contaminated waters enter the lake: the Chamberonne River, the outlet of the Flon stream at 10 m depth, and the outlet of the WWTP at 30 m depth (i.e., below point V14) (Figure 1 and Table 1). The injection into the river was performed by directly pouring the phage suspension into the center of the river; the injections into the lake were performed from R/V La Licorne (Institute FA Forel) using a pump and hosepipe.

Water Sampling. Ten sampling campaigns for bacteriological analyses were performed between March 2005 and February 2006. Samples were taken at points V1 through V23 at a depth of 2 m, except for points V20a,b close to the shoreline, where the sampling depth was 1 m. Additional samples were taken at the three sites where contaminated waters enter the bay (Figure 1). During the tracer tests, water samples for bacteriophage analyses were taken at 14 of these points in the lake and, additionally, at the drinking water pumping station. The sampling campaign in the lake lasted for 2 days and included up to five samples at each point, while the monitoring at the pumping station lasted for 3 days and included eight samples. Water samples were kept at 4 °C in the dark, as solar irradiation and an increased temperature strongly impairs the survival of the phages (*18*).

Bacteriophage Detection. The phages were analyzed by the double layer assay (*19*) within 24 h after sampling. An unfiltered water sample (2 mL) to be tested for the presence of a given bacteriophage was mixed with 400 μL of an

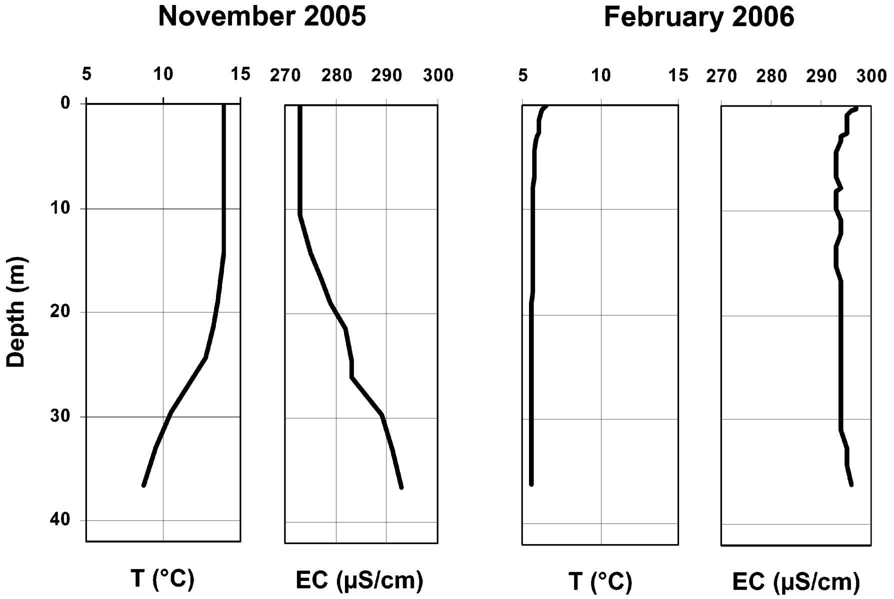

FIGURE 2. Depth profiles of temperature (*T*) and electrical conductivity (EC) at point V14c recorded during the two multi-tracer tests.

TABLE 1. Types and Quantities of Bacteriophages (Number of Phages in 10 L) Introduced for the Tracer Tests

phage type	quantity	injection point	Depth (m)	date and time
H4/4	5.55×10^{13}	Flon	10	11/7/2005 09:40
H6/1	6.97×10^{13}	WWTP	30	11/7/2005 09:25
H40/1	3.15×10^{15}	Chamberonne	surface	11/7/2005 09:32
H4/4	6.99×10^{13}	Flon	10	2/21/2006 10:20
H6/1	1.14×10^{14}	WWTP	30	2/21/2006 09:55
H40/1	1.09×10^{15}	Chamberonne	surface	2/21/2006 09:54

exponentially growing culture of the corresponding host bacterium. After an infection time of 5 min for H4/4 (15 min for H6/1 and H40/1), 3−4 mL of molten agar (0.6%) with a temperature of 45−50 °C was added, mixed, and immediately poured into a Petri dish already containing a first layer of marine PY agar (1%) that supports growth of the host bacterium. The phages present in a sample formed clearing zones in the developing uniform bacterial carpet in the upper agar layer. After 16−24 h at 22 °C, lysis plaques were counted. Water samples with high phage titers were serially diluted in PBS (pH 7) that contained additionally one drop of 1 M $CaCl_2$ plus 1 mL of 1 M $MgCl_2$ per 500 mL of PBS (*18*, *20*). The detection limit of this method is 0.5 PFU/mL. All analyses were performed on three parallel Petri dishes. Although some salt tolerant freshwater bacteria could potentially grow on this nonselective medium, contaminants were never observed as they are suppressed by the fast growing host strains.

Batch Experiments. To check the stability of bacteriophages in lake water, batch experiments were carried out in the laboratory. Water from the bay was collected prior the tracing experiments, spiked with a mix of all three phages (final concentration ca. 100 PFU/mL each), and incubated at 19 °C under dim light and gentle agitation. Samples were withdrawn regularly, and the phage titer was determined in triplicates with the method described previously.

Bacteriological Analysis. Water samples were examined for FIB, including total coliforms (TC), *Escherichia coli*, and enterococci (ENT). Analyses were performed by the Lausanne water supply, according to the Swiss standard methods. Most results are single determinations and are expressed as colony forming units (CFU) per 100 mL; concentration differences between triplicates of selected samples were typically within 30%.

Results and Discussion

Limnological Conditions. The profiles measured during the first tracer test displayed a thermocline with an inflection point at 25 m depth (Figure 2). The temperature in the epilimnion was 14 °C and steadily dropped to 9 °C in the hypolimnion, accompanied by an increase in the EC. The profiles measured during the second tracer test show a well-mixed water column; the EC is nearly constant, and the temperature decreases only slightly from 6.5 °C at the surface to 5.6 °C at depth.

During the first tracer test, when wind was weakly blowing from the southwest, the drifters were predominantly displaced eastwards, suggesting a clockwise water circulation in the bay, although only the northern, near-shore part of this circulation was observed. Conversely, the drifters moved mainly in a northwestern direction during the second tracer experiment, although wind was again blowing from the southwest at 10 km/h. Displacement velocities ranged between 1 and 8 cm/s. The velocities of the deep-water currents were variable but generally lower than those of the surface currents. During the first tracer test, the direction of the deep-water currents followed an Ekman spiral, whereby the currents at 11 m depth were reversed by 180° relative to the surface current. However, during the second tracer test in February 2006, the deep-water currents followed the general direction of the surface currents.

Bacteriological Water Quality. High FIB levels were found at the three sites where contaminated waters enter the bay: often more than 10^5 enterococci, 10^6 *E. coli*, and 10^7 total coliforms per 100 mL in the outlet of the WWTP and *E. coli* contents exceeding 10^5/100 mL in the Flon outlet and 10^4/100 mL in the Chamberonne River. The microbial contamination in lake water samples collected at 2 m depth is lower

TABLE 2. Summary of Multi-Tracer Results[a]

		November 2005			February 2006		
		bacteriophage type			bacteriophage type		
sampling point		H4/4	H6/1	H40/1	H4/4	H6/1	H40/1
V-1	**t_1 (h)**	24.5	ND[b]	48.6	25.3	5.6	5.6
	C_{max} (PFU/mL)	0.9	ND	0.7	0.5	1.60×10^6	5.5
V-2	**t_1 (h)**	ND	ND	ND	25.4	5.5	5.5
	C_{max} (PFU/mL)	ND	ND	ND	0.5	3.27×10^6	6.5
V-3	**t_1 (h)**	ND	ND	24.8	ND	25.9	25.9
	C_{max} (PFU/mL)	ND	ND	2.0	ND	5.83×10^6	14.5
V-4	**t_1 (h)**	ND	ND	ND	25.6	5.8	5.9
	C_{max} (PFU/mL)	ND	ND	ND	0.5	5.53×10^6	9.5
V-7	**t_1 (h)**	ND	ND	ND	ND	ND	ND
	C_{max} (PFU/mL)	ND	ND	ND	ND	ND	ND
V-9	**t_1 (h)**	ND	ND	25.1	25.9	26.3	50.8
	C_{max} (PFU/mL)	ND	ND	0.5	5.0	5.0	1.0
V-10	**t_1 (h)**	ND	ND	5.0	50.3	26.3	ND
	C_{max} (PFU/mL)	ND	ND	37.8	1.5	6.5	ND
V-11	**t_1 (h)**	48.7	ND	3.0	25.8	26.2	2.6
	C_{max} (PFU/mL)	0.2	ND	58.5	2.0	1.67×10^6	5.0
V-14	**t_1 (h)**	ND	ND	24.1	25.6	5.9	5.9
	C_{max} (PFU/mL)	ND	ND	13.6	1.5	3.30×10^7	66.7
V-20[c]	**t_1 (h)**	ND	ND	2.3	25.7	6.1	2.0
	C_{max} (PFU/mL)	ND	ND	454.7	1.0	1.73×10^7	1.83×10^7
V-23	**t_1 (h)**	24.3	ND	ND	ND	26.4	ND
	C_{max} (PFU/mL)	0.7	ND	ND	ND	8.5	ND
pumping station					ND	ND	ND

[a] For each sampling point, t_1 is the time after injection where a phage type has first been detected, and C_{max} is the maximum concentration observed at this point during the whole course of the tracing experiment (mean of triplicates). [b] ND: not detected. [c] Including points V-20a–d.

but still exceeds frequently the Swiss legal limits of 0/100 mL for drinking water. The highest levels of *E. coli*, often 10^2 to 10^4/100 mL or even more, were generally found in samples taken near the known contaminant sources. However, even samples taken at points several kilometers further to the west, relatively close to the drinking water pumping station, show *E. coli* contents of up to 60/100 mL. The measured FIB levels are highly variable in time and space (i.e., concentrations differing by several orders of magnitude were frequently observed in spatially and temporally close samples). For example, during the first tracer test, *E. coli* levels of 17 000/100 mL were detected at V20a, while a sample taken at the neighboring V20d contained only 52/100 mL.

Stability of the Bacteriophages. In batch experiments with nonsterile lake water, all three phage types remained detectable for more than 300 h, thus sufficiently long for the tracer tests, which lasted about 60 h. In November 2005, the number of infectious phages decreased with half-life times of about 70 h for H40/1, 120 h for H4/4, and 140 h for H6/1 (Figure S1, Supporting Information). Interestingly, the decay rates were strongly reduced in water collected in February 2006 under identical incubation conditions. After 75 h, nearly 100% of H6/1, 90% of H40/1, and about 60% of H4/4 were still infectious (data not shown). While the temperature is typically considered as a main controlling factor for phage inactivation (*21*), these data suggest that it depends additionally on the overall biological activity in the lake water (e.g., proteolytic enzymes, grazing by protozoa), which was likely higher at the end of fall than in winter. The batch incubations were performed at 19 °C, which was higher than measured in the lake (Figure 2). The determined decay rates thus represent maximum estimates.

Tracing Results. The results of the two multi-tracer tests show some similarities but also some notable differences that illustrate the role of the thermal state of the lake for water circulation and pathogen transport (Table 2). During both experiments, H40/1 phages, which were injected into the Chamberonne River, spread rapidly in the bay. Already after 2–3 h, concentrations >100 PFU/mL were measured near the mouth of the river (V20a–d) in November; even $>10^7$ PFU/mL were detected at these points during the second tracer experiment. In the following days, lower concentrations of H40/1 were detected at most points in the bay, whereby the general propagation pattern nicely corresponded with the surface currents (Figure 3). In both tracer tests, H40/1 phages also appeared at V1, the sampling point closest to the drinking water pumping station. Matching with a suspected clockwise surface water circulation during the first tracer experiment, it took a rather long 48.6 h to arrive there. In February when the surface current circulation was reversed and the traveling distance was shorter, H40/1 was detected already after 5.6 h. Also, the results of the H4/4 phages, which were injected at 10 m depth near the outlet of the Flon drainage pipe, were similar for both experiments. Although H4/4 was found at fewer points and at lower concentrations, it displayed a similar propagation pattern as H40/1. This reduced detection was partly due to the lower amount of injected H4/4 phages (Table 1) but additionally a result of a less efficient propagation of the tracer in the bay since the Flon stormwater outlet was not active during both tracing experiments. The most significant differences were found for H6/1 phages, which were injected near the WWTP outlet pipe at 30 m depth. In November, when the lake was stratified, not a single phage was detected in any of the 54 samples that were taken during the whole experiment. This finding confirms that the well-developed thermocline at 25 m depth acted as a highly effective barrier preventing vertical transport from the hypolimnion to the epilimnion. During the February experiment, when the water column was mixed, H6/1 phages appeared only 5–6 h after injection at several sampling points. The concentrations often exceeded 10^6 and sometimes 10^7 PFU/mL, indicating a rapid upwelling of water from the depth. The phages first arrived at sampling sites in the central and western part of the bay (V1, V2, V4, V14, and V20), confirming the northwestward, counter-clockwise circulation that was also recognized by the surface drifters.

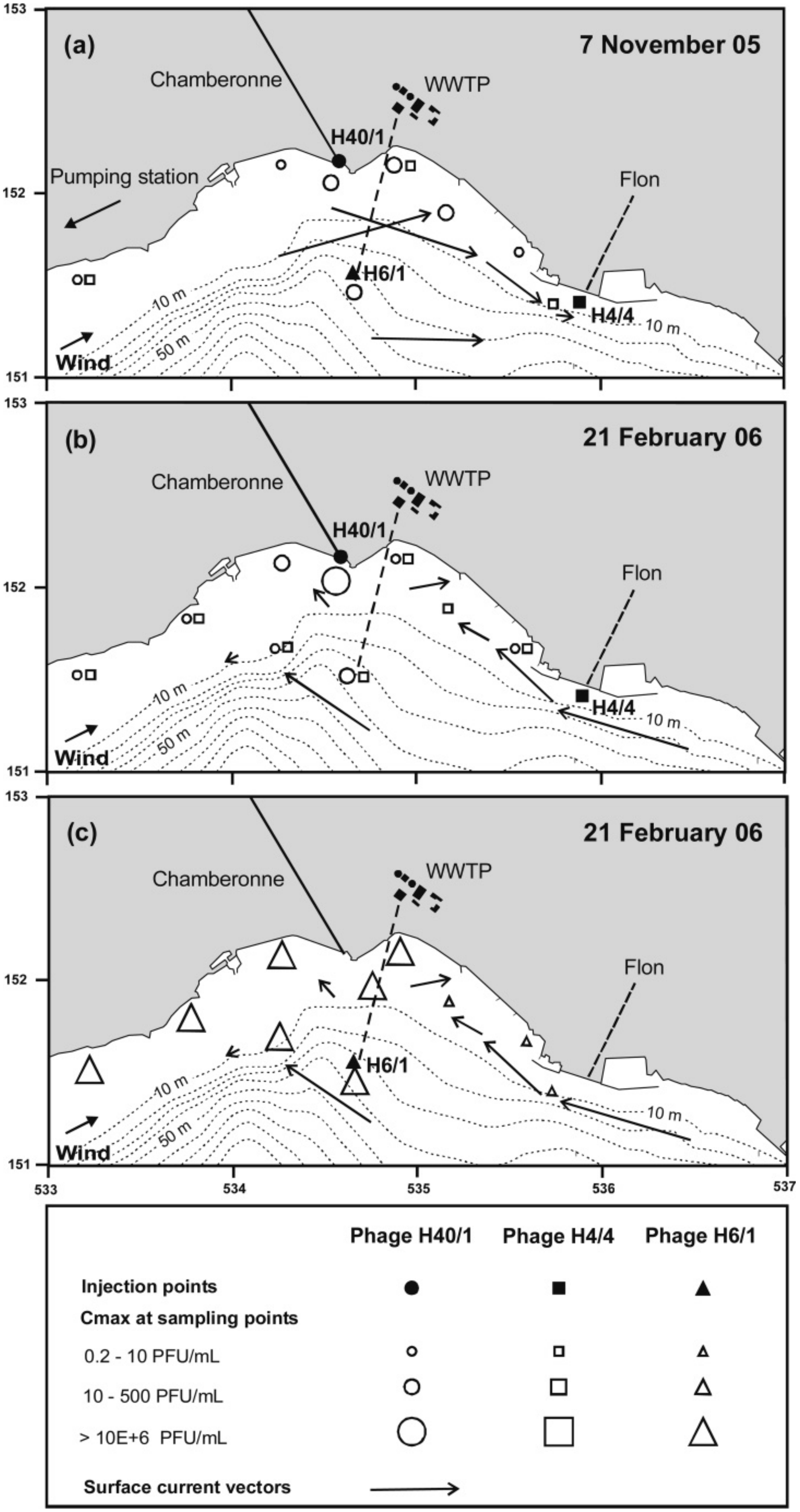

FIGURE 3. Multi-tracer results (maximum phage concentrations $C_{\max}$) and surface-drifter displacement vectors: (a) H40/1 and H4/4, November 2005, (b) H40/1 and H4/4, February 2006, and (c) H6/1, February 2006. No H6/1 phages were detected during the November 2005 experiment.

Transport Processes. Extreme differences in concentrations were observed in temporally and spatially close samples, indicating that the distribution of the phages in the bay was highly heterogeneous (Supporting Information, Tables S1 and S2). For example, during the February experiment, no H6/1 phages were observed at point V4 in the samples taken 2.2, 7.5, and 50.4 h after the injection, while high levels were detected in samples collected after 5.8 h (5.5×10^6 PFU/mL) and 26.0 h (5.53×10^6 PFU/mL). Similarly, during the third sampling campaign of this tracer test, 1.62×10^7 PFU/mL H6/1 were detected at V14, while the results from all other points were negative. These findings suggest that the propagation of the phages in the bay does not occur by homogeneous mixing processes, but in the form of plumes with sharp boundaries, which are laterally displaced with the overall water current while permanently changing their form. Therefore, it is not possible to interpolate between different measurement points (i.e., draw iso-concentration lines). For the same reason, it is not possible to interpolate in time between measured concentrations at a given sampling point. The heterogeneous transport processes established by the bacteriophage tracer test are consistent with the observed spatial and temporal variability of the FIB levels. A similar observation was made by Wanninkhof and coworkers (*22*), who used SF_6 to trace the discharge plume of a point source in coastal waters. Within the first 20 km from the outfall, highly variable SF_6 surface concentrations were measured, while they decreased monotonically with distance afterward.

Consequences for Water Protection. Although no phages were detected at the drinking water pumping station, it is important to note that five of the six bacteriophage injections that were performed during the two multi-tracer tests resulted in positive detections at the westernmost sampling point (V1), at only 1.5 km distance from the pumping station. During the February experiment, the H6/1 phages arrived at V1 at high concentrations of 1.60×10^6 PFU/mL after only 5.6 h. These results indicate that it is possible that the continuous input of partly persistent pathogens via the known contamination sources can actually reach the area of the drinking water pumping station. The results of the tracer tests further confirm that contaminated waters, mainly from the WWTP outlet, can rapidly reach the surface when the lake is thermally homogeneous, spread in the bay, and also impact its tourist beaches, although this impact is highly variable in time and space.

Advantages and Drawbacks of the Methodology. Tracer tests provide direct evidence and quantitative information on the propagation of contaminants. In the present case, the combination of tracer tests and limnological methods made it possible to obtain a more complete picture of water circulation and contaminant transport and to identify the influence of the thermal state of the lake on these processes. The advantages of using marine bacteriophages as tracers include their absence in freshwater, their low detection limits, the simplicity of producing large quantities, and their invisibility and non-harmfulness to all organisms other than their specific bacterial host (*18*). There is no significant interference with other phage types and tracer substances such as fluorescent dyes. The stability of phages in the environment is limited; thus, they do not accumulate and the background level remains negligible, which makes repeated tracing experiments at the same site possible. On the other hand, the limited persistence also restricts the duration of sampling campaigns to a few days or weeks, although this was not a critical issue for this study. Furthermore, the detection of phages relies on discrete water sampling and subsequent analysis in the laboratory within 24 h, while methods of continuous on-site detection are not available. In conclusion, marine bacteriophages can be recommended as tracers to study the transport of contaminants, particularly viruses and other pathogens. Their properties make them favorable for multi-tracer tests in freshwater systems with high dilutions, such as lakes, and when visible coloring and other environmental impacts must be avoided.

Acknowledgments

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Supporting Information Available

Stability tests of bacteriophages in lake water as well as complete data from the two tracer tests illustrating the temporal and spatial variability in the distribution of phages in the Bay of Vidy. This material is available free of charge via the Internet at http://pubs.acs.org.

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