Published in final edited form as:
J Virol Methods. 2021 October ; 296: 114245. doi:10.1016/j.jviromet.2021.114245.

Performance evaluation of a dead-end hollowfiber ultrafiltration method for enumeration of somatic and F+ coliphage from recreational waters

Asja Korajkic*, **Brian R. McMinn**, **Michael P. Herrmann**, **Adin C. Pemberton**, **Julie Kelleher**, **Kevin Oshima**, **Eric N. Villegas**
United States Environmental Protection Agency, Office of Research and Development, USA

Abstract

Dead-end hollow fiber ultrafiltration combined with a single agar layer assay (D-HFUF-SAL) has potential use in the assessment of sanitary quality of recreational waters through enumeration of coliphage counts as measures of fecal contamination. However, information on applicability across a broad range of sites and water types is limited. Here, we tested the performance of D-HFUF-SAL on 49 marine and freshwater samples. Effect of method used to titer the spiking suspension (SAL versus double agar layer [DAL]) on percent recovery was also evaluated. Average somatic coliphage recovery (72 % ± 27) was significantly higher ($p < 0.0001$) compared to F+ (53 % ± 19). This was more pronounced for marine ($p \leq 0.0001$) compared to freshwaters ($p = 0.0134$). Neither method affected somatic coliphage, but DAL (28 % ± 12) significantly ($p < 0.0001$) underestimated F + coliphage recoveries compared to SAL (53 % ± 19). Overall, results indicate that, while D-HFUF-SAL performed well over a wide variety of water types, F + coliphage recoveries were significantly reduced for marine waters suggesting that some components unique to this habitat may interfere with the assay performance. More importantly, our findings indicate that choice of spike titer method merits careful consideration since it may under-estimate method percent recovery.

Corresponding author at: 26W Martin Luther King Jr. Drive, Cincinnati, OH, USA. korajkic.asja@epa.gov (A. Korajkic).

Author statement
AK: Conceptualization, Methodology, Formal analysis, Investigation, Writing-Original Draft, Writing-Review and Editing, Visualization, Supervision.
BRM: Conceptualization, Methodology, Investigation, Writing-Original Draft, Writing-Review and Editing.
MPH: Investigation, Writing-Original Draft, Writing-Review and Editing.
ACP: Investigation, Writing-Original Draft, Writing-Review and Editing.
JK: Investigation, Writing-Original Draft, Writing-Review and Editing.
KO: Conceptualization, Writing-Original Draft, Writing-Review and Editing.
EVN: Conceptualization, Writing-Original Draft, Writing-Review and Editing.

Disclaimer
The United States Environmental Protection Agency through its Office of Research and Development funded and managed the research described here. It has been subjected to Agency's administrative review and approved for publication. The views expressed in this article are those of the author(s) and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Declaration of Competing Interest
The authors report no declarations of interest.

Keywords

Somatic and F+ coliphage; Dead-end hollowfiber ultrafiltration; Single agar layer; Double agar layer; Method performance

1. Introduction

Recreational waters can become contaminated with untreated or partially treated wastewater via many different pathways, including regulated and unregulated discharges such as combined sewer overflows, sanitary sewer overflows and faulty infrastructure. As a result, recreational water users can become exposed to a myriad of different pathogens. Pathogenic viruses have been identified as common etiologic agents in recreational waterborne disease outbreaks (Begier et al., 2008; Sinclair et al., 2009; Yoder et al., 2008). However, direct enumeration of viral pathogens from recreational water is difficult since their concentrations tend to be low, infectious viral assays (e.g. cell culture) are tedious and time consuming, and molecular approaches (e.g. qPCR, RT-qPCR) do not discriminate between infectious and non-infectious viral particles. Because it is not feasible to test recreational waters for the presence of all viral (and other) pathogens, fecal indicator bacteria (FIB), such as *Escherichia coli* and enterococci have been used in the assessment of sanitary quality of recreational waters for over a century. However, one of the many caveats associated with FIB is different fate and transport characteristics compared to those of viral pathogens (Ferguson et al., 2003; Korajkic et al., 2018; McMinn et al., 2017a), highlighting the need for viral indicator for recreational water quality monitoring.

Bacteriophages that infect *E. coli* (somatic and F + coliphages) have been proposed as indicators of fecal contamination in recreational waters and as potential surrogates for viral pathogens because they satisfy many of the criteria for fecal indicator organisms while sharing key morphological and structural characteristics with those of pathogenic viral species (King et al., 2011).

Coliphages may also better mimic fate and transport characteristics of viral pathogens in natural and built environments compared to FIB (Cole et al., 2003; Meschke and Sobsey, 2003; Montazeri et al., 2015; Nappier et al., 2006; Yahya et al., 2015). For example, coliphages have been consistently detected in wastewater (Korajkic et al., 2020; Nappier et al., 2019) and their removal rates through wastewater treatment processes are similar to those of infectious enteric viruses (McMinn et al., 2017a). While there are limited data on fate and transport of coliphages and pathogenic viruses in the environment (Korajkic et al., 2019), some reports indicate that the decay of coliphages in ambient water is similar to that of noroviruses (Bae and Schwab, 2008), polioviruses (Skraber et al., 2004), and adenoviruses (McMinn et al., 2020).

However, one of the potential issues with using somatic and F + coliphages for recreational water quality assessment is that their concentrations in ambient waters may be too low (Boehm et al., 2009; McMinn et al., 2017a; Ortega et al., 2009; Viau et al., 2011) to detect using conventional methods that typically assay 1–100 ml water samples (American Public Health Association, 2005a, b; American Public Health Association, 2005c, d;

American Public Health Association, 2005e; International Organization for Standardization, 1995, 2000; United States Environmental Protection Agency, 2001), necessitating sample concentration. Based on earlier reports demonstrating the utility of hollowfiber ultrafiltration (HFUF) to concentrate a broad range of microbial targets (Hill et al., 2007; Hill et al., 2005; Kuhn and Oshima, 2001, 2002; Leskinen et al., 2009; Morales-Morales et al., 2003; Mull et al., 2012; Rhodes et al., 2016), we utilized the HFUF in the dead-end set-up (D-HFUF) and combined it with a single agar layer (SAL) enumeration procedure to develop a method (D-HFUF-SAL) that allows for the analysis of the entire eluate volume, equivalent to one-liter water samples per coliphage type (somatic and F+) (McMinn et al., 2017b).

The D-HFUF-SAL method was compared and found superior to other methods capable of analyzing similar sample volumes (McMinn et al., 2018). While method performance was satisfactory, yielding 64–80 % and 48–67 % recovery for somatic and F + coliphage, respectively, it was tested on relatively small number of samples from limited geographic regions (McMinn et al., 2017b; Wanjugi et al., 2018) including four lake water samples (William H. Harsha Lake, OH; Lake Erie, OH; Lake Michigan, IN; Lake Michigan, WI;), four river water samples (Ohio River, OH; Cuyahoga River, OH; Trail Creek, IN; Oak Creek, WI) and a single marine water sample (Morgan Beach Park, FL). In order to determine the ability of D-HFUF-SAL to concentrate and enumerate coliphages from a wide variety of recreational water types, a more thorough evaluation is needed.

Another important factor potentially influencing coliphage recovery in recreational waters is the spike titer procedure. Spike titer refers to the process of quantifying the spiking material in order to estimate the percent recovery of a given method. Some researchers use SAL (McMinn et al., 2017b; Wanjugi et al., 2018), while others recommend the use of a double agar layer (DAL) method to estimate percent recovery of the D-HFUF-SAL procedure (United States Environmental Protection Agency, 2018a). In our earlier studies, we obtained relatively uniform percent recovery estimates when using SAL to enumerate the spike titer (i.e. 48–80 %) (McMinn et al., 2017b; Wanjugi et al., 2018). However, utilization of DAL for the enumeration of spike titer material generated more variable percent recovery estimates (9–401 %), irrespective of the coliphage or the water type (United States Environmental Protection Agency, 2018a). The percent recovery in a sample is an important quality assurance metric because it establishes the acceptable method performance criteria. At the moment, no direct comparisons between DAL and SAL are available and it remains unknown whether using one spike titer method or the other significantly affects percent recovery determination.

Here we evaluate the performance of the D-HFUF-SAL method on 49 recreational water sample collected from marine and freshwater sites across the United States. Furthermore, we investigate the potential effect of various physical-chemical parameters on percent recoveries and assess whether spike titer method selection (e.g. SAL versus DAL) influences percent recovery. Findings suggest that D-HFUF-SAL is a robust methodology across water types, but that the spike titer approach can influence measurement of method performance.

2. Materials and methods

2.1 Sample collection and physical-chemical measurements

Forty-nine ambient water samples (~ 12 L each) were collected from lakes (n = 20), rivers/creeks/canals (n = 17) and marine/estuarine environments (n = 12) spanning 18 states across the United States (Table 1). All samples were collected in sterile containers and stored at 4 °C until processing (typically within one week). Samples that required shipping, were collected in the same manner and shipped overnight, on ice to US EPA Cincinnati, OH, laboratories. Prior to start of each experiment, a series of physical-chemical measurements were recorded. A ProDSS Multiparameter Water Quality Meter (YSI Incorporated, Yellow Springs, OH) was used to measure, specific conductance (μS/cm), pH and turbidity (FNU). Nitrate and phosphate concentrations (mg/L NO_3–N and PO_4, respectively) were measured using Hach Phosphate and a Nitrate Colorimeter II (Hach, Loveland, CO) according to manufacturer instructions. A handheld refractometer (Extech instruments, Waltham, MA) was used to obtain salinity readings (‰). List of sampling sites and accompanying physical and chemical measurements are summarized in Table 1.

2.2. Wastewater derived coliphage spike preparation

Primary treated wastewater was collected from a local wastewater treatment plant and transported to the laboratory on ice (holding time < 6 h). Ten mL of wastewater (per coliphage type) was passed through a 0.22 μm polyethersulfone syringe filter and added to 90 ml bacterial host culture in mid-log growth phase (*E. coli* CN-13 [ATCC 700609] for somatic and *E. coli* Famp [ATCC 700891] for F + coliphage, American Type Culture Collection [ATCC], Manassas, VA) grown in tryptic soy broth (TSB) containing the appropriate antibiotics (100 μg/ml nalidix acid for *E. coli* CN-13, 15 μg/ml streptomycin/ampicillin for *E. coli* Famp [Sigma Aldrich, St. Louis, MO]). Wastewater spiked host cultures were incubated overnight at 37 °C. The following day, cultures were centrifuged at 3500 x g for 5 min, and the supernatant was filtered through a 0.22 μm syringe filter. Decimal dilution series of the filtered supernatant were created using sterile TSB as recommended by standard methods (United States Environmental Protection Agency, 2018a, b) and initial coliphage concentrations were determined by both DAL and SAL assays.

2.3. Coliphage spiking procedure

Upon the receipt of a sample, indigenous background somatic and F + coliphage levels were enumerated to determine whether spiking with wastewater derived coliphages was needed. If background indigenous coliphage concentrations were ≥ 100 Plaque Forming Units per Liter (PFU/L), these input levels were deemed sufficient to estimate percent recovery and these samples were not spiked with the wastewater-derived coliphage. Instead, 100 ml aliquots per coliphage type of unconcentrated sample were assayed the day of the experiment using SAL and served in lieu of spiking material to determine percent recovery. If background indigenous coliphage concentrations were below this threshold, samples were spiked with wastewater derived coliphage.

For the spiking procedure, triplicate, two-liter bulk water aliquots for each sample were brough to room temperature and vortexed to mix for 10 min. Each two-liter water

sample was spiked by adding 1 ml of wastewater derived somatic coliphage spike and 1 ml of wastewater derived F + coliphage spike (target concentration ≥ 100 PFU per coliphage type). Spiked samples were vortexed for an additional 15 min prior to D-HFUF filtration. In addition, background indigenous coliphage levels for all spiked samples were determined using D-HFUF-SAL the day of the experiment, and these coliphage background concentrations were subtracted from coliphage concentrations obtained from spiked, concentrated sample to minimize artificial deflation of percent recoveries as recommended by standard methods (United States Environmental Protection Agency, 2018a, b). Finally, somatic and F + coliphage spiking material was enumerated the day of the experiment, using both SAL and DAL methodology.

2.4. Coliphage concentration by D-HFUF and enumeration using SAL and DAL

D-HFUF procedure was performed as previously described (McMinn et al., 2017b). Briefly, two-liter water sample was passed through 15S Asahi Kasei Rexeed ultrafilters (Dial Medical Supply, Chester Springs, PA) using a peristaltic pump (Masterflex L/S Easy Load, Cole Parmer, Vernon Hills, IL) set at 300 rpm (approximately 850 mL/min). Filters were eluted by circulating 200 ml of elution solution (0.01 % Tween 80, 0.01 % sodium hexametaphosphate, 0.001 % Antifoam Y-30 [Sigma-Aldrich, St. Louis, MO]) in clockwise, counter-clockwise and finally clockwise directions for 1 min each (McMinn et al., 2017b). The resulting filter eluate was evenly divided (approximately 100 mL per coliphage type); therefore, the final volume per coliphage type that was enumerated by SAL was equivalent to one liter.

The SAL method was performed as previously described (McMinn et al., 2017b; United States Environmental Protection Agency, 2018b). Briefly, 100 ml of either filter eluate, unconcentrated water sample or TSB containing 1 ml of coliphage spike(s) was added to 100 ml of molten 2X tryptic soy agar (TSA) (Fisher Scientific, Waltham, MA), followed by 10 mL of appropriate *E. coli* host(s), 2 ml of an appropriate antibiotic(s) and 0.5 ml 4 M $MgCl_2$ (Sigma Aldrich, St. Louis, MO), plated over five large (150 mm diameter) petri dishes, and incubated at 37 °C for 16–18 h.

The DAL method was used only for the analyses of coliphage spikes and it was performed as previously described (United States Environmental Protection Agency, 2018a, b). Briefly, 500 μL of wastewater derived spike was added to 5 ml of semi-solid TSA (0.7 % agar) containing appropriate antibiotic(s), followed by the addition of 100 μL of appropriate host culture. The resulting mixture was plated on a single 1X TSA (containing the appropriate antibiotics) petri dish (100 mm diameter) and incubated at 37 °C for 16–18 h. The volumetric difference in spike processing between the two methods (e.g. 1 ml for SAL versus 500 μL for DAL) were accounted for prior to percent recovery calculations. Lastly, during each experiment, negative controls (sample substituted with sterile TSB) and media sterility checks (plates containing agar and antibiotics with no sample) yielded no detectable PFU, indicating absence of contamination in coliphage reagents used.

2.5. Data analysis

Percent recoveries were calculated using the following formula: PR = (CS-CUS)/S x 100 where: PR = percent recovery; CS = coliphage concentrations in spiked sample (i.e. number of organisms recovered); CUS = coliphage concentrations in un-spiked sample (i.e. background) and S = coliphage concentrations in spiked material (i.e. input number of organisms). Percent recovery data was arcsine square root transformed prior to statistical analyses. For direct comparisons of spike concentrations between the two methods, coliphage concentrations were $\log_{10}$ transformed before analyses. GraphPad Prism software (version 8.1.2 for Windows, GraphPad Software, San Diego, CA) was used for all statistical analyses. Two-tailed paired *t*-tests were used to assess the effect of the water type and spike titer method on percent recoveries and for direct comparisons of spike concentrations. One-way analysis of variance with Tukey's multiple comparison test was used to evaluate the effect of water type on percent recoveries, while Pearson product moment correlation tests were used to determine the relationships between percent recoveries and physical-chemical measurements. A two-tailed unpaired *t*-test was used to determine whether there was a statistically significant difference in somatic coliphage percent recoveries between background coliphage levels and wastewater derived spikes ($\alpha = 0.05$).

3. Results

3.1. Physical-chemical data and background coliphage concentrations

Physical-chemical parameters are summarized in Table 1. Measurements ranged from 100.5 – 52 806 μS/cm (specific conductance), 7.15–9.18 (pH), 0.7–94 FNU (turbidity), 0–35 ‰ (salinity), 0–12.1 mg/L (nitrates) and 0–2.82 mg/L (phosphates). Background indigenous somatic and F + coliphage were present in 61.2 % (n = 30) and 36.7 % (n = 18) samples, respectively and ranged from 1 to 2300 per 100 ml PFU for somatic and from 1 to 19 per 100 ml PFU for F + coliphage (data not shown). All 49 samples required the addition of a wastewater-derived F + coliphage spike, while 35 samples required a somatic coliphage spike. Of the 14 samples not requiring somatic coliphage spikes, 71.4 % (n = 10) were from rivers/creeks/canals and 28.6 % (n = 4) were from lakes. There was no statistically significant difference in percent recoveries for somatic coliphage when background concentrations versus wastewater derived spikes were used (two-tailed unpaired *t*-test; $p = 0.2721$).

3.2. Effect of water type on D-HFUF-SAL method performance

Average percent recoveries of somatic coliphage (71.28 ± 26.85 %) were significantly higher ($p < 0.0001$) compared to F+ (52.66 ± 19.01 %). Distributions of average percent recoveries (calculated with SAL titer spike approach) across all water types, as well as percent recoveries by the water type are summarized in Fig. 1. Average somatic coliphage recoveries across three sample replicates ranged from 10.07 ± 29.26 % (Hilo Bay, HI) to 136 ± 25.32 % (Wilkson Bayou, LA). Lowest average F+ coliphage recoveries were also recorded for Hilo Bay, HI sample (5.30 ± 6.28 %), with the highest average recovery (98.58 ± 35.33 %) observed for the Lake Erie, OH sample. When examined by water type, average percent recoveries for somatic coliphage (lakes: 73.60 ± 19.37 %; rivers/creeks/canals: 64.69 ± 19.37 %; marine/estuarine: 76.66 ± 39.08 %) were not significantly different ($p = 0.1309$).

Average percent recoveries for F + coliphage in lakes (59.72 ± 18.27 %) and rivers/creeks/canals (54.19 ± 16.74 %) were also similar ($p = 0.2311$), but significantly higher ($p \leq 0.0001$) compared to marine waters (38.77 ± 15.94 %). Average somatic coliphage percent recoveries were higher than F + for all water types, but this difference was more pronounced for marine/estuarine waters ($p \leq 0.0001$) compared to rivers/creeks/canals ($p = 0.0134$).

3.3 Correlations of D-HFUF-SAL percent recoveries with physical-chemical parameters

The results of Pearson product moment correlation tests examining relationships between percent recoveries obtained with the SAL titer spike approach and various physical chemical parameters are presented in Table 2. Percent recoveries of somatic and F + coliphage were significantly correlated ($R^2 = 0.454$, $p < 0.0001$). The only other statistically significant ($p = 0.015$) finding for somatic coliphage was a decrease in percent recoveries associated with increased turbidity ($R^2 = - 0.201$, $p = 0.015$). F + coliphage percent recoveries exhibited two statistically significant relationships; namely decreased percent recoveries associated with increased specific conductance and salinity (specific conductance: $R^2 = -0.431$, $p < 0.0001$, salinity: $R^2 = -0.428$, $p < 0.0001$). Of note, specific conductance and salinity measurements were also significantly correlated (R2 = 0.998, $p < 0.0001$).

3.4. Effect of spike titer method on percent recoveries

Comparison of spike titer procedures was performed for all samples requiring the addition of wastewater derived coliphage spikes. The method used to titer the spike had a considerable effect on percent recovery determinations, especially for F + coliphage (Fig. 2). A direct comparison of spike concentrations analyzed by both methods did not yield statistically significant ($p = 0.6126$) results for somatic coliphage, but it did for F+ ($p < 0.0001$) with DAL yielding significantly higher concentration levels compared to SAL. Therefore, use of DAL to titer somatic coliphage slightly overestimated the average recovery compared to SAL (75.73 ± 34.70 % DAL versus 71.28 ± 26.85 % SAL), but the difference was not statistically significant ($p = 0.0846$). In contrast, the use of DAL to titer F + coliphage severely underestimated percent recoveries for all samples compared to SAL (28.08 ± 12.04 % DAL versus 52.66 ± 19.01 % SAL) and this difference was statistically significant ($p < 0.0001$).

4. Discussion

Application of D-HFUF-SAL to monitor somatic and F + coliphage concentrations in ambient waters is a promising approach for recreational water quality assessment, but to date the performance of the method has been evaluated on a relatively small number of samples representing limited geographic areas. In this study, we evaluated the ability of the D-HFUF-SAL to recover either spiked, wastewater derived or indigenous somatic and F + coliphage from 49 freshwater and marine/estuarine water samples collected from 18 different geographic regions. Given the enormous diversity of somatic and F + coliphages belonging to multiple taxonomic groups including *Myoviridae, Siphoviridae, Podoviridae, Microviridae, Leviviridae, Inoviridae* and *Tectiviridae* (Burbano-Rosero et al., 2011; King et al., 2011; Korf et al., 2019), we opted to utilize coliphages indigenous to municipal wastewater, as opposed to singular strains (e.g. MS2, ΦX-174) because this approach

is likely to establish a more accurate understanding of the method performance in the intended setting. Finally, we evaluated the effect of the method utilized to enumerate coliphage spiking preparation (i.e. SAL versus DAL), because an earlier report (United States Environmental Protection Agency, 2018a) suggested that the choice of procedure may affect the D-HFUF-SAL performance.

Overall, somatic coliphage recoveries from the diverse subset of ambient waters tested were 64.7 ± 22.6 %, 73.6 ± 19.4 % and 76.7 ± 39.1 % for lakes, rivers/creeks/canals and marine/estuarine samples, respectively with an average percent recovery of 71.3 ± 26.9 %. These percent recoveries closely resembled previously reported values observed during the initial development of the D-HFUF-SAL method (62 ± 16 %, 79 ± 14 % and 72 ± 25 % for lakes, rivers and marine waters, respectively) (McMinn et al., 2017b). Evaluation of the method in the Great Lakes Basin yielded similar percent recoveries, ranging from 63.7 ± 15.5%–78.6 ± 6.8 % for lake water and 69.6 ± 12%–80.2 ± 5.5 % for river water (Wanjugi et al., 2018), indicating robustness of the method across different water types.

Turbidity was the only physical-chemical parameter measured in this study that significantly impacted somatic coliphage recoveries. Similar to some earlier reports (McMinn et al., 2017b), we noted an inverse relationship with somatic coliphage percent recoveries, suggesting that particulate matter from water samples can interfere with recovery. However, unlike previous findings (Mull and Hill, 2012), there was no statistically significant relationship between F + coliphage recoveries and turbidity measurements. Some of the potential explanations for the seeming discrepancy are considerably lower turbidity measurements observed in this study (13.2 ± 18.6 NTU), compared to earlier report (92 ± 7.9 NTU) (Mull and Hill, 2012) and possibly the origin of the F + coliphage (i.e. multitude of wastewater derived strains used in this study versus singular MS2 strain utilized in previous report).

When examined by water type, percent recoveries for F + coliphage in lakes (59.72 ± 18.27 %) and rivers/creeks/canals (54.19 ± 16.74 %) were slightly lower than what has been observed previously (63 ± 6% lakes; 62 ± 15 % river) (McMinn et al., 2017b), but were within the ranges reported during the Great Lakes Basin study (58.6 ± 6.2%–66.7 ± 11.7 % for lakes, 48.1 ± 8.1%–65.1 ± 9.0 % for rivers) (Wanjugi et al., 2018). However, F + coliphage recoveries from marine/estuarine water in the current study (38.77 ± 15.94 %, n = 12) were considerably lower than previously reported (72 ± 21 %, n = 1), which could be, at least partly, attributed to a smaller sample size (McMinn et al., 2017b). Salinity and specific conductance were the only two physical-chemical parameters measured that affected F + coliphage percent recoveries in current study, suggesting that D-HFUF-SAL method performance for this coliphage group may be reduced in marine waters as compared for freshwater. Earlier studies indicated that elevated salinity (and accompanying specific conductivity) inherent to estuarine/marine waters can result in a more rapid inactivation of viruses, including coliphages, as compared to freshwater (Boehm et al., 2019; Korajkic et al., 2019). Indeed, inactivation of F + coliphages was shown to be significantly faster in marine water as compared to lake water (Jeanneau et al., 2012), and direct comparison of somatic and F + coliphage decay characteristics in marine waters indicated that the latter was inactivated significantly faster (Wanjugi et al., 2016). However, it is not clear whether

the limited contact time in the current study was sufficient to cause the observed reduction in percent recoveries. Additionally, divalent salts have been found to cause aggregation of F + coliphages (Mylon et al., 2010; Nguyen et al., 2011) which could contribute to underestimates of PFUs in culture-based assays.

While performance of large volume concentration methods, such as D-HFUF, has been documented for FIB (Leskinen et al., 2009; Leskinen and Lim, 2008) and some viral pathogens (Cuevas-Ferrando et al., 2021; Grant et al., 2011) from marine and estuarine waters, relatively little is known about the its performance for the concentration and enumeration of coliphages in the same water types. In addition to faster inactivation rates and potential aggregation issues which seemingly disproportionally affected F + coliphages in this study, a recent report indicated that coralline and silica sand can interfere with performance of other methods in these environments (Seruge et al., 2019). Interestingly, the lowest average percent recoveries for both coliphage types (≤ 10 %) were observed for Hilo Bay, HI, a sampling site with relatively high silicic acid concentrations (Wiegner et al., 2017), suggesting that sand may also interfere with the performance of coliphage methods in marine and estuarine waters.

While determining the mechanisms responsible for these interactions was beyond the scope of the present study, an earlier report suggesting that as many as 99 % of viruses in coastal waters could be attached to naturally occurring colloids and particles (Finiguerra et al., 2011) lends credence to supposition that adsorption to sand particles can affect the performance of coliphage culture-based assays by reducing their infective capability. For example, a recent study showed that coralline beach sand effectively removed 99.99 % of MS2 bacteriophage, along with adenoviruses, echoviruses, noroviruses and rotaviruses from septic tank effluents, likely through adsorption (Humphries et al., 2020). Furthermore, a controlled laboratory study suggests that the inactivation of both MS2 and ΦX-174 coliphages in the presence of quartz/silica sand is more rapid as compared to controls in the absence of sand (Chrysikopoulos and Aravantinou, 2012), suggesting that a similar mechanism may be possible at some recreational beaches. Considering the uneven performance of the method across different water types and paucity of estuarine/marine samples (< 25 %) in this study, future research efforts should focus on a more thorough assessment of method performance in estuarine/marine waters, identification of the underlying mechanisms responsible for the reduced percent recoveries and strategies to improve method performance in these water types such as acidification (Seruge et al., 2019) or some other approach.

Another study objective was to determine whether the method employed to enumerate the spiking suspension (DAL versus SAL) influences coliphage percent recovery determinations. Spike titer is used to identify acceptable percent recovery ranges for particular sample types to ensure accurate measurement of a target microorganism. Head to head comparisons of recreational water samples in this study indicate that while the DAL method yielded elevated recoveries for somatic coliphage relative to SAL (e.g. 75.73 ± 34.70 % DAL versus 71.28 ± 26.85 % SAL), the difference between spike titer methods was not significant ($p = 0.6126$), suggesting equivalent performance of both methods for this coliphage type. However, when DAL was used to titer F + coliphage spikes, percent

recovery estimates were significantly reduced compared to the SAL method (e.g. 28.08 ± 12.04 % DAL versus 52.66 ± 19.01 % SAL). An explanation for this observation could be due to procedural variations between these methods (SAL versus DAL) that differentially influence somatic compared to F + coliphage types, such as density of agar, ratio of bacterial host to coliphage spike, as well as the addition of $MgCl_2$ during the SAL assay. While it was outside of the scope of the current study to determine the underlying cause for variations in method performance, this important finding merits careful consideration and warrants further research, as it may lead to inaccurate assessment of method performance.

5. Conclusions

Viral pathogens are important etiologic agents of recreational waterborne illness outbreaks, highlighting the need for viral indicators for recreational water quality assessment. In this study, we tested the performance of somatic and F + coliphage counts using D-HFUF-SAL on 49 marine and freshwater samples collected nationwide. Furthermore, we also evaluated the effect of spike titer method (SAL versus DAL) on percent recoveries of somatic and F + coliphages. Our results were generally comparable to what has been previously reported, indicating good performance of the method across a wide variety of water types. However, our data suggests that the environmental water matrix can have an important impact on method performance as F + coliphage percent recoveries were significantly lower in marine waters, possibly due to the presence of coralline or silica sands. These findings merit further research to improve method performance in marine waters, possibly through additional sample manipulations in the form of acidification. Furthermore, our data indicates that the choice of spike titer procedures requires careful consideration, as it can substantially impact evaluations of method performance, especially for F + coliphage.

Acknowledgements

We would like to thank many of our collaborators, colleagues, friends and family members who graciously agreed to collect and send us the water samples. Without your help this project would not have been possible.

Funding

This work was supported by the United States Environmental Protection Agency.

References

American Public Health Association, A.W.W.A, Water Environment Federation, 2005a. 9224 B. Somatic coliphages assay. In: Eaton AD, Clesceri LS, Rice EW, Greenberg AE (Eds.), Standard Methods for the Examination of Water and Wastewater. American Public Health Association, American Water Works Association, Water Environment Federation, Washington, D.C.

American Public Health Association, A.W.W.A, Water Environment Federation, 2005b. 9224 C. Male-specific coliphage assay using Escherichia coli famp. In: Eaton AD, Clesceri LS, Rice EW, Greenberg AE (Eds.), Standard Methods for the Examination of Water and Wastewater. American Public Health Association, American Water Works Association, Water Environment Federation, Washington, D. C.

American Public Health Association, A.W.W.A, Water Environment Federation, 2005c. 9224 D. Male-specific coliphage assay using Salmonella typhimurium WG49. In: Eaton AD, Clesceri LS, Rice EW, Greenberg AE (Eds.), Standard Methods for the Examination of Water and Wastewater. American Public Health Association, American Water Works Association, Water Environment Federation, Washington, D. C.

American Public Health Association, A.W.W.A, Water Environment Federation, 2005d. 9224 E. Single agar layer method. In: Eaton AD, Clesceri LS, Rice EW, Greenberg AE (Eds.), Standard Methods for the Examination of Water and Wastewater. American Public Health Association, American Water Works Association, Water Environment Federation, Washington, D.C.

American Public Health Association, A.W.W.A, Water Environment Federation, 2005e. 9224 F. Membrane filter method. In: Eaton AD, Clesceri LS, Rice EW, Greenberg AE (Eds.), Standard Methods for the Examination of Water and Wastewater. American Public Health Association, American Water Works Association, Water Environment Federation, Washnigton, D.C.

Bae J, Schwab KJ, 2008. Evaluation of murine norovirus, feline calicivirus, poliovirus, and MS2 as surrogates for human norovirus in a model of viral persistence in surface water and groundwater. Appl. Environ. Microbiol. 74, 477–484. [PubMed: 18065626]

Begier EM, Oberste MS, Landry ML, Brennan T, Mlynarski D, Mshar PA, Frenette K, Rabatsky-Ehr T, Purviance K, Nepaul A, Nix WA, Pallansch MA, Ferguson D, Cartter ML, Hadler JL, 2008. An outbreak of concurrent echovirus 30 and coxsackievirus A1 infections associated with sea swimming among a group of travelers to Mexico. Clin. Infect. Dis. 47, 616–623. [PubMed: 18637756]

Boehm AB, Yamahara KM, Love DC, Peterson BM, McNeill K, Nelson KL, 2009. Covariation and photoinactivation of traditional and novel indicator organisms and human viruses at a sewage-impacted marine beach. Environ. Sci. Technol. 43, 8046–8052. [PubMed: 19924921]

Boehm AB, Silverman AI, Schriewer A, Goodwin K, 2019. Systematic review and meta-analysis of decay rates of waterborne mammalian viruses and coliphages in surface waters. Water Res. 164, 114898.

Burbano-Rosero EM, Ueda-Ito M, Kisielius JJ, Nagasse-Sugahara TK, Almeida BC, Souza CP, Markman C, Martins GG, Albertini L, Rivera IN, 2011. Diversity of somatic coliphages in coastal regions with different levels of anthropogenic activity in Sao Paulo State, Brazil. Appl. Environ. Microbiol. 77, 4208–4216. [PubMed: 21531842]

Chrysikopoulos CV, Aravantinou AF, 2012. Virus inactivation in the presence of quartz sand under static and dynamic batch conditions at different temperatures. J. Hazard. Mater. 233, 148–157. [PubMed: 22819478]

Cole D., Long SC., Sobsey MD., 2003. Evaluation of F RNA and DNA coliphages as source-specific indicators of fecal contamination in surface waters. Appl. Environ. Microbiol. 69, 6507–6514. [PubMed: 14602607]

Cuevas-Ferrando E, Perez-Cataluna A, Allende A, Guix S, Randazzo W, Sanchez G, 2021. Recovering coronavirus from large volumes of water. Sci. Total Environ. 762, 143101.

Ferguson C, Husman AMD, Altavilla N, Deere D, Ashbolt N, 2003. Fate and transport of surface water pathogens in watersheds. Crit. Rev. Environ. Sci. Technol. 33, 299–361.

Finiguerra MB, Escribano DF, Taylor GT, 2011. Light-independent mechanisms of virion inactivation in coastal marine systems. Hydrobiologia 665, 51–66.

Grant AA, Jakob E, Richard J, Garver KA, 2011. Concentration of infectious aquatic rhabdoviruses from freshwater and seawater using ultrafiltration. J. Aquat. Anim. Health 23, 218–223. [PubMed: 22372250]

Hill VR, Polaczyk AL, Hahn D, Narayanan J, Cromeans TL, Roberts JM, Amburgey JE, 2005. Development of a rapid method for simultaneous recovery of diverse microbes in drinking water by ultrafiltration with sodium polyphosphate and surfactants. Appl. Environ. Microbiol. 71, 6878–6884. [PubMed: 16269722]

Hill VR, Kahler AM, Jothikumar N, Johnson TB, Hahn D, Cromeans TL, 2007. Multistate evaluation of an ultrafiltration-based procedure for simultaneous recovery of enteric microbes in 100-liter tap water samples. Appl. Environ. Microbiol. 73, 4218–4225. [PubMed: 17483281]

Humphries B, Weaver L, Burbery L, Webber J, Morgan L, Gregor J, 2020. Microbial pathogen removal from domestic effluent using coral sand in Kiribati. J. Appl. Microbiol. 128, 1208–1220. [PubMed: 31846171]

International Organization for Standardization, 1995. Water Quality- Detection and Enumeration of Bacteriophages-part 1: Enumeration of F-specific Bacteriophages, Geneva, Switzerland.

International Organization for Standardization, 2000. Water Quality- Detection and Enumeration of Bacteriophages- Part 2: Enumeration of Somatic Coliphages, Geneva, Switzerland.

Jeanneau L, Solecki O, Wery N, Jarde E, Gourmelon M, Communal PY, Jadas-Hecart A, Caprais MP, Gruau G, Pourcher AM, 2012. Relative decay of fecal indicator bacteria and human-associated markers: a microcosm study simulating wastewater input into seawater and freshwater. Environ. Sci. Technol. 46, 2375–2382. [PubMed: 22236067]

King AMQ, Adams MJ, Carstens EB, Lefkowitz WJ, 2011. Virus Taxonomy: Classification and Nomenclature of Viruses. Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, London, UK.

Korajkic A, McMinn BR, Harwood VJ, 2018. Relationships between microbial indicators and pathogens in recreational water settings. Int. J. Environ. Res. Public Health 15.

Korajkic A, Wanjugi P, Brooks L, Cao Y, Harwood VJ, 2019. Persistence and decay of fecal microbiota in aquatic habitats. Microbiol. Mol. Biol. Rev. MMBR 83.

Korajkic A, McMinn B, Herrmann MP, Sivaganesan M, Kelty CA, Clinton P, Nash MS, Shanks OC, 2020. Viral and bacterial fecal indicators in untreated wastewater across the contiguous United States exhibit geospatial trends. Appl. Environ. Microbiol.

Korf IHE, Meier-Kolthoff JP, Adriaenssens EM, Kropinski AM, Nimtz M, Rohde M, van Raaij MJ, Wittmann J, 2019. Still something to discover: novel insights into Escherichia coli phage diversity and taxonomy. Viruses 11.

Kuhn RC, Oshima KH, 2001. Evaluation and optimization of a reusable hollow fiber ultrafilter as a first step in concentrating Cryptosporidium parvum oocysts from water. Water Res. 35, 2779–2783. [PubMed: 11456179]

Kuhn RC, Oshima KH, 2002. Hollow-fiber ultrafiltration of Cryptosporidium parvum oocysts from a wide variety of 10-L surface water samples. Can. J. Microbiol. 48, 542–549. [PubMed: 12166681]

Leskinen SD, Lim DV, 2008. Rapid ultrafiltration concentration and biosensor detection of enterococci from large volumes of Florida recreational water. Appl. Environ. Microbiol. 74, 4792–4798. [PubMed: 18515479]

Leskinen SD, Harwood VJ, Lim DV, 2009. Rapid dead-end ultrafiltration concentration and biosensor detection of enterococci from beach waters of Southern California. J. Water Health 7, 674–684. [PubMed: 19590135]

McMinn BR, Ashbolt NJ, Korajkic A, 2017a. Bacteriophages as indicators of faecal pollution and enteric virus removal. Lett. Appl. Microbiol. 65, 11–26. [PubMed: 28304098]

McMinn BR., Huff EM., Rhodes ER., Korajkic A., 2017b. Concentration and quantification of somatic and F + coliphages from recreational waters. J. Virol. Methods 249, 58–65. [PubMed: 28843788]

McMinn BR., Rhodes ER., Huff EM., Wanjugi P., Ware MM., Nappier SP., Cyterski M., Shanks OC., Oshima K., Korajkic A., 2018. Comparison of somatic and F + coliphage enumeration methods with large volume surface water samples. J. Virol. Methods 261, 63–66. [PubMed: 30096350]

McMinn BR, Rhodes ER, Huff EM, Korajkic A, 2020. Decay of infectious adenovirus and coliphages in freshwater habitats is differentially affected by ambient sunlight and the presence of indigenous protozoa communities. Virol. J. 17, 1. [PubMed: 31906972]

Meschke JS., Sobsey MD., 2003. Comparative reduction of Norwalk virus, poliovirus type 1, F + RNA coliphage MS2 and Escherichia coli in miniature soil columns. Water Sci. Technol. 47, 85–90.

Montazeri N, Goettert D, Achberger EC, Johnson CN, Prinyawiwatkul W, Janes ME, 2015. Pathogenic enteric viruses and microbial indicators during secondary treatment of municipal wastewater. Appl. Environ. Microbiol. 81, 6436–6445. [PubMed: 26162869]

Morales-Morales HA, Vidal G, Olszewski J, Rock CM, Dasgupta D, Oshima KH, Smith GB, 2003. Optimization of a reusable hollow-fiber ultrafilter for simultaneous concentration of enteric bacteria, protozoa, and viruses from water. Appl. Environ. Microbiol. 69, 4098–4102. [PubMed: 12839786]

Mull B, Hill VR, 2012. Recovery of diverse microbes in high turbidity surface water samples using dead-end ultrafiltration. J. Microbiol. Methods 91, 429–433. [PubMed: 23064261]

Mylon SE, Rinciog CI, Schmidt N, Gutierrez L, Wong GC, Nguyen TH, 2010. Influence of salts and natural organic matter on the stability of bacteriophage MS2. Langmuir 26, 1035–1042. [PubMed: 19775143]

Nappier SP, Aitken MD, Sobsey MD, 2006. Male-specific coliphages as indicators of thermal inactivation of pathogens in biosolids. Appl. Environ. Microbiol. 72, 2471–2475. [PubMed: 16597945]

Nappier SP, Hong T, Ichida A, Goldstone A, Eftim SE, 2019. Occurrence of coliphage in raw wastewater and in ambient water: a meta-analysis. Water Res. 153, 263–273. [PubMed: 30735956]

Nguyen TH, Easter N, Gutierrez L, Huyett L, Defnet E, Mylon SE, Ferri JK, Viet NA, 2011. The RNA core weakly influences the interactions of the bacteriophage MS2 at key environmental interfaces. Soft Matter 7, 10449–10456.

Ortega C, Solo-Gabriele HM, Abdelzaher A, Wright M, Deng Y, Stark LM, 2009. Correlations between microbial indicators, pathogens, and environmental factors in a subtropical estuary. Mar. Pollut. Bull. 58, 1374–1381. [PubMed: 19464704]

Rhodes ER, Huff EM, Hamilton DW, Jones JL, 2016. The evaluation of hollow- fiber ultrafiltration and celite concentration of enteroviruses, adenoviruses and bacteriophage from different water matrices. J. Virol. Methods 228, 31–38. [PubMed: 26562058]

Seruge J, Wong M, Noble RT, Blackwood AD, Moravcik PS, Kirs M, 2019. Application of a rapid qPCR method for enterococci for beach water quality monitoring purposes in Hawaii: loss of DNA during the extraction protocol due to coral sands. Mar. Pollut. Bull. 149, 110631.

Sinclair RG, Jones EL, Gerba CP, 2009. Viruses in recreational water-borne disease outbreaks: a review. J. Appl. Microbiol. 107, 1769–1780. [PubMed: 19486213]

Skraber S, Gassilloud B, Schwartzbrod L, Gantzer C, 2004. Survival of infectious Poliovirus-1 in river water compared to the persistence of somatic coliphages, thermotolerant coliforms and Poliovirus-1 genome. Water Res. 38, 2927–2933. [PubMed: 15223287]

United States Environmental Protection Agency, 2001. Method 1602: Male-specific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure, Washington, D.C..

United States Environmental Protection Agency, 2018a. Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure, Washington, D.C..

United States Environmental Protection Agency, 2018b. Method 1643: Male-specific (F+) and Somatic Coliphage in Secondary (No Disinfection) Wastewater by the Single Agar Layer (SAL) Procedure, Washington, D.C..

Viau EJ, Goodwin KD, Yamahara KM, Layton BA, Sassoubre LM, Burns SL, Tong HI, Wong SH, Lu Y, Boehm AB, 2011. Bacterial pathogens in Hawaiian coastal streams–associations with fecal indicators, land cover, and water quality. Water Res. 45, 3279–3290. [PubMed: 21492899]

Wanjugi P, Sivaganesan M, Korajkic A, Kelty CA, McMinn B, Ulrich R, Harwood VJ, Shanks OC, 2016. Differential decomposition of bacterial and viral fecal indicators in common human pollution types. Water Res. 105, 591–601. [PubMed: 27693971]

Wanjugi P., Sivaganesan M., Korajkic A., McMinn B., Kelty CA., Rhodes E., Cyterski M., Zepp R., Oshima K., Stachler E., Kinzelman J., Kurdas SR., Citriglia M., Hsu FC., Acrey B., Shanks OC., 2018. Incidence of somatic and F+ coliphage in Great Lake Basin recreational waters. Water Res. 140, 200–210. [PubMed: 29715644]

Wiegner TN, Edens CJ, Abaya LM, Carlson KM, Lyon-Colbert A, Molloy SL, 2017. Spatial and temporal microbial pollution patterns in a tropical estuary during high and low river flow conditions. Mar. Pollut. Bull. 114, 952–961. [PubMed: 27866724]

Yahya M, Hmaied F, Jebri S, Jofre J, Hamdi M, 2015. Bacteriophages as indicators of human and animal faecal contamination in raw and treated wastewaters from Tunisia. J. Appl. Microbiol. 118, 1217–1225. [PubMed: 25689071]

Yoder JS, Hlavsa MC, Craun GF, Hill V, Roberts V, Yu PA, Hicks LA, Alexander NT, Calderon RL, Roy SL, Beach MJ, Centers for Disease, C. and Prevention, 2008. Surveillance for waterborne disease and outbreaks associated with recreational water use and other aquatic facility-associated health events–United States, 2005–2006. Morbidity and mortality weekly report. Surveill. Summaries 57, 1–29.

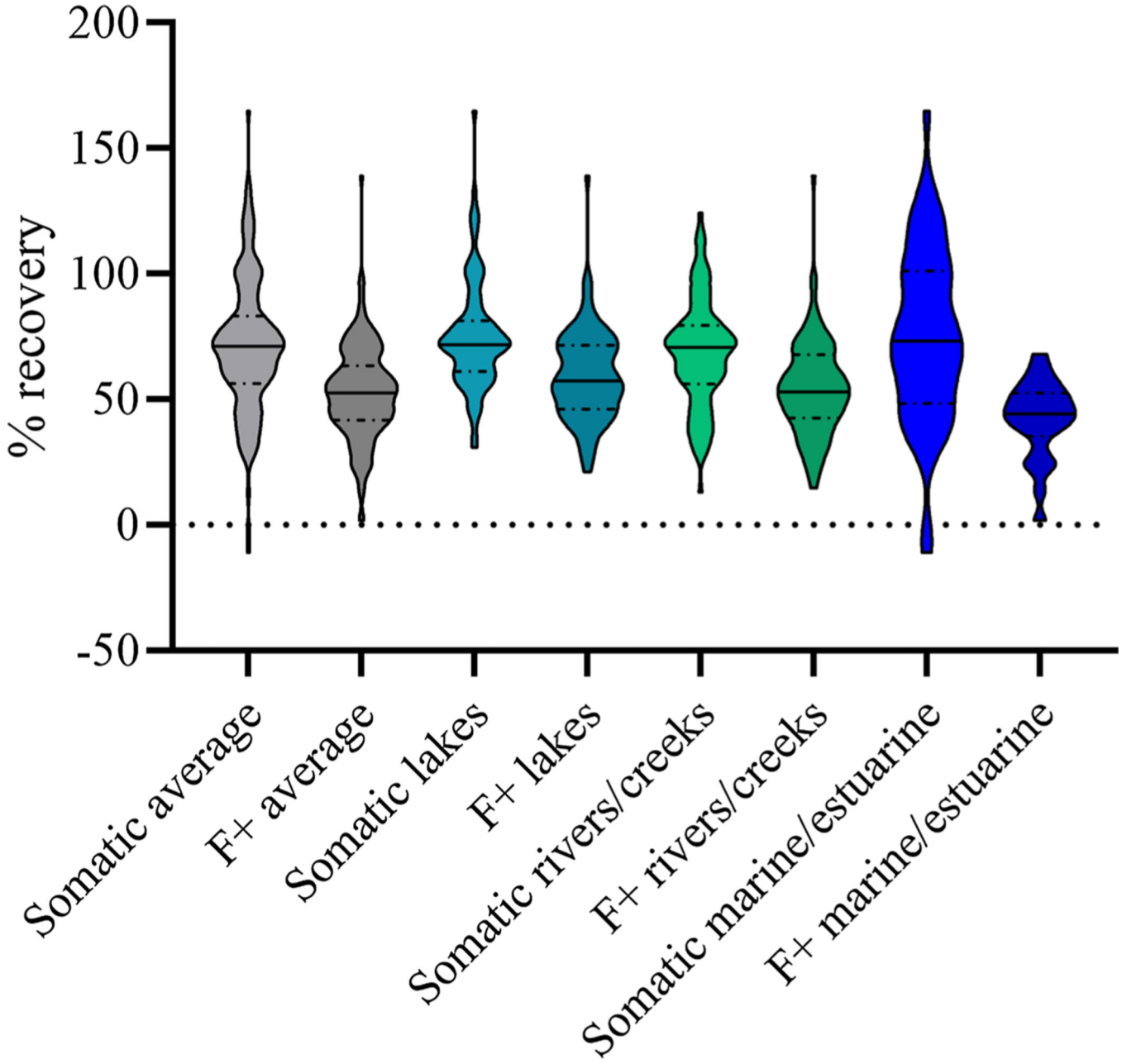

Fig. 1.

Distribution of average and water type specific percent recoveries for somatic and F + coliphage. The solid line represents the median, while dashed lines represent quartiles.

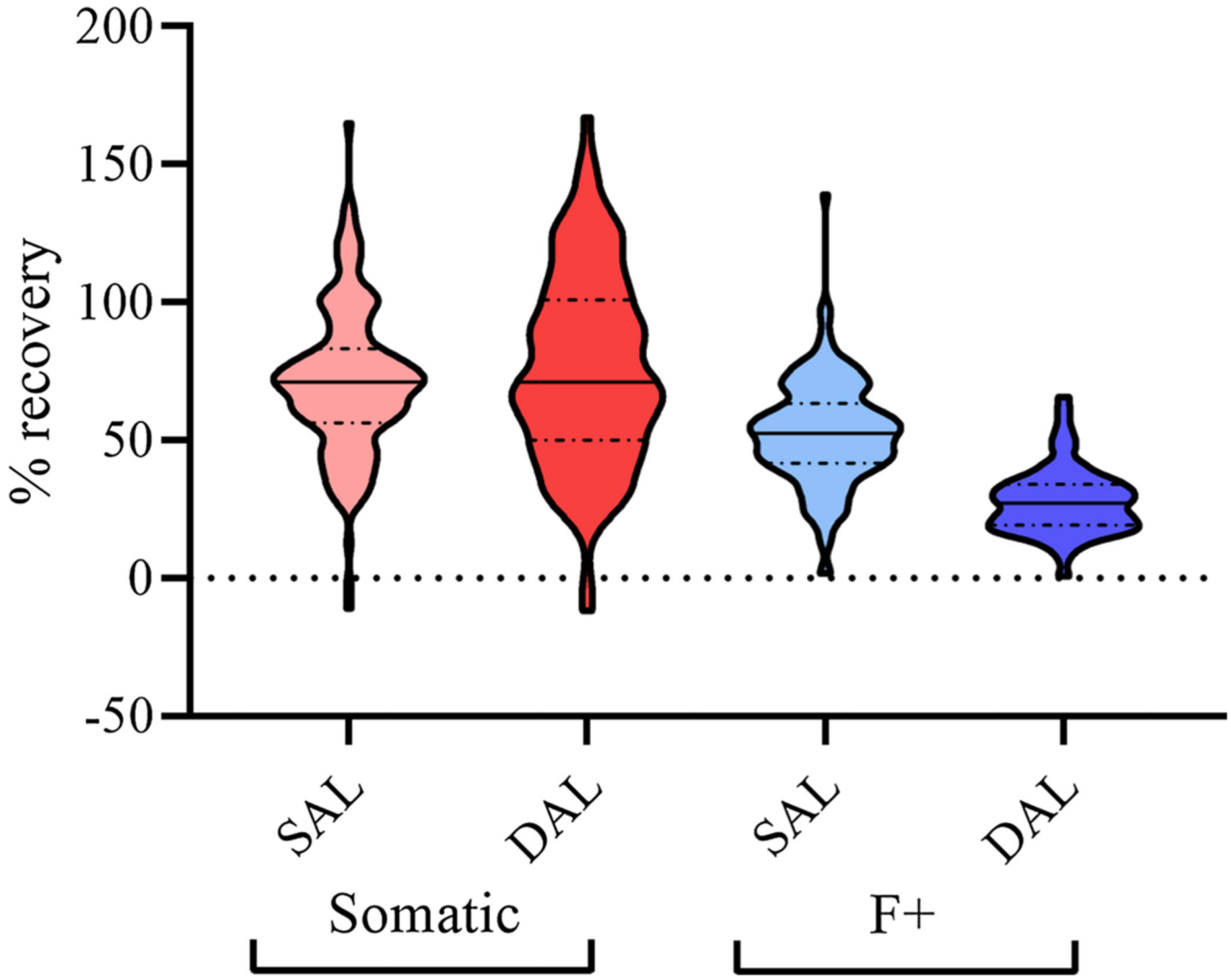

Fig. 2.

The effect of spike titer method (SAL versus DAL) on average somatic and F + coliphage recoveries. The solid line represents the median, while dashed lines represent quartiles.

J Virol Methods. Author manuscript; available in PMC 2022 April 05.

Table 1

List of sampling sites and associated and physical chemical data.

Site	Specific conductance (µS/cm)	pH	Turbidity (FNU)	Nitrate (mg/L NO_3-N)	Phosphate (mg/L PO_4)	Salinity (‰)
Lake Harsha, OH *	204.5	8.48	34.8	0.70	0.63	0.00
Williamstown Lake, OH *	288.5	8.15	9.30	0.00	0.46	0.00
Winton Lake, OH *	577.0	7.52	25.9	12.1	0.15	0.00
Lake Kincaid, KY *	410.5	8.12	7.60	1.30	0.17	0.00
Cave Run Lake, KY	151.9	8.14	6.10	1.20	0.12	0.00
Holmes Lake, NE	401.3	7.86	43.4	0.00	0.05	0.00
Oak Lake, NE	2571	7.54	60.1	0.00	0.00	0.00
Bowling Lake, NE	896.0	7.94	14.5	0.10	0.03	0.00
Joe Pool Lake, TX	428.7	7.26	94.0	0.00	0.08	0.00
Lake Erie, OH	304.6	8.14	3.50	1.00	0.00	0.00
Quarry Lake, WI	618.0	8.76	1.40	0.56	0.19	0.00
Lake Michigan (Lakeside Park), WI	306.2	8.49	4.10	0.40	0.22	0.00
Lake Michigan (White Fish Dunes), WI	298.3	7.98	0.80	1.10	0.05	0.00
Lake Michigan (Bailey's Harbor), WI	302.0	7.71	3.00	0.80	0.00	0.00
Lake Michigan (Frank E. Murphy Park), WI	319.2	7.71	0.70	0.50	0.01	0.00
Lake Mona, MI	456.0	7.74	1.80	0.80	0.12	0.00
Grapevine Lake, TX	372.0	7.30	2.80	0.00	0.09	0.00
Lake Waxahachie, TX	267.0	8.05	2.90	1.10	0.13	0.00
Lake Huron, MI	370.6	7.98	4.30	0.20	0.18	0.00
Brookville Lake, IN	410.8	8.05	3.40	0.90	0.24	0.00
Little Miami River, OH *	380.0	7.90	50.4	0.00	0.32	0.00
Ohio River, KY *	307.3	7.47	47.3	0.50	0.21	0.00
Licking River, KY *	165.1	7.85	24.8	0.20	0.46	0.00
Great Miami River, OH *	696.0	8.19	5.30	1.50	0.27	0.00
North Oconee River, GA *	103.6	7.40	19.0	0.60	0.10	0.00
Middel Oconee River, GA *	100.5	7.15	15.8	0.50	0.10	0.00
Morgan Creek, NC	165.8	7.86	4.30	0.10	0.00	0.00

Site	Specific conductance (µS/cm)	pH	Turbidity (FNU)	Nitrate (mg/L NO_3-N)	Phosphate (mg/L PO_4)	Salinity (‰)
Northeast Creek, NC	576.0	7.32	11.1	1.30	2.82	0.00
James River, MO*	363.4	7.62	5.50	0.90	0.20	0.00
Hudson River, NY*	331.2	8.43	1.90	0.70	0.12	0.00
Antelope Creek, NE*	529.0	7.35	4.70	1.20	0.77	0.00
Root River, WI*	963.0	7.73	14.7	0.90	0.80	0.00
Rio Grande, NM	525.0	7.91	33.6	0.00	0.33	0.00
Elm Fork of Trinity River, TX	407.0	7.73	4.90	0.90	0.11	0.00
Amarillo Canal Site#1, AZ	936.0	8.20	3.00	1.00	0.16	0.00
Amarillo Canal Site#2, AZ	600.0	9.18	3.90	0.00	0.14	0.00
Mississippi River, LA	488.8	8.09	20.7	0.00	0.40	0.00
Quiet Water Beach, FL	38,330	8.13	6.30	1.00	0.04	24.0
Santa Rosa Dunes, FL	45,091	7.90	3.30	0.60	0.09	32.0
Taylor’ s Creek, NC	35,748	7.47	1.50	0.60	0.24	22.0
Newport Beach, CA	52,220	7.83	0.70	1.20	0.11	33.6
Mandalay Beach, CA	52,806	7.86	2.00	0.90	0.12	35.0
Morgan Park, FL	51,231	7.70	6.00	0.10	0.09	35.0
Bellair Causeway, FL	48,820	7.82	7.40	0.10	0.27	33.0
Clam Bay, WA	48,562	7.25	0.70	0.00	0.25	31.0
Honolii, HI	47,131	7.87	0.80	0.00	0.06	29.4
Hilo Bay, HI	44,239	7.69	11.0	0.90	0.14	29.5
Wilkson Bayou, LA	17,880	7.24	6.57	0.70	0.28	9.00
Lake Pontchartrain, LA	4183	7.83	2.70	0.30	0.17	2.09

*Denotes samples where background, indigenous somatic coliphage levels, rather than the wastewater derived preparations were used for spike for percent recovery calculations.

Table 2

Pearson product moment correlation between SAL coliphage percent recoveries and physical chemical data. The upper right portion of the table represents two-tailed **p** values, while the bottom left portion shaded in gray represents R^2 values. Bolded cells denote statistically significant relationships.

	Somatic coliphage	F+ coliphage	Specific conductance (*μ*S/cm)	pH	Turbidity (FNU)	Nitrate (mg/L NO_3-N)	Phosphate (mg/L PO_4)	Salinity (‰)
Somatic coliphage		**< 0.0001**	0.276	0.770	**0.015**	0.287	0.212	0.279
F+ coliphage	**0.454**		**<0.0001**	0.221	0.221	0.183	0.789	**<0.0001**
Specific conductance (μS/cm)	0.091	**-0.431**		0.053	**0.002**	0.314	0.121	**<0.0001**
pH	-0.024	0.102	-0.160		**0.003**	0.208	0.048	0.070
Turbidity (FNU)	**-0.201**	0.102	**-0.257**	**-0243**		0.921	0.844	**0.002**
Nitrate (mg/L NO_3-N)	0.089	0.111	-0.084	-0.104	-0.008		0.707	0.314
Phosphate (mg/L PO_4)	-0.104	-0.022	-0.129	-0.164	0.016	0.031		0.118
Salinity (‰)	0.091	**-0.428**	**0.998**	-0.150	**-0258**	-0.084	-0.129	

J Virol Methods. Author manuscript; available in PMC 2022 April 05.