From autosamplers to passive samplers: Microbial monitoring in stormwater constructed wetlands

Des échantillonneurs automatiques aux échantillonneurs passifs : Surveillance microbiologique dans les zones humides artificielles de traitement des eaux pluviales

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RÉSUMÉ

La surveillance microbiologique dans les zones humides construites pour les eaux pluviales est essentielle pour la qualité de l'eau et la santé publique. Cette étude a évalué des membranes passives électro-négatives par rapport à des échantillonneurs automatiques composites pour la détection du CrAssphage à l'entrée et à la sortie d'une zone humide lors d'événements pluvieux. La régression logistique n'a montré aucune différence significative de fréquence de détection (p>0,05), mais la durée de déploiement influençait fortement la performance des échantillonneurs passifs. Des déploiements plus longs (48-96 h) ont amélioré la détection et fourni des signaux quantitatifs cohérents. La régression quantile a révélé une absorption linéaire significative avec un taux d'accumulation estimé à 7,44 $\mathrm{mL \cdot j^{-1}}$ (IC 95 %). Les concentrations pondérées dans le temps (TWAC) ont montré que des déploiements supérieurs à 48 h donnent des estimations fiables des concentrations dans la colonne d'eau. Cette étude confirme que les échantillonneurs passifs offrent une approche pratique et intégrée dans le temps pour la surveillance microbiologique des zones humides, avec un potentiel prometteur pour le calcul rétroactif des TWAC, nécessitant toutefois une validation supplémentaire.

ABSTRACT

Effective microbial monitoring in stormwater-constructed wetlands is essential for water quality and public health protection. This study evaluated passive electronegative membranes against automatic composite samplers for detecting CrAssphage at the inlet and outlet of the stormwater constructed wetland during rainfall events. Logistic regression showed no significant difference in detection frequency (p>0.05), but deployment duration strongly influenced passive sampler performance. Longer deployments (48-96 h) improved detection and provided consistent quantitative signals. Quantile regression revealed a significant linear uptake with an estimated accumulation rate of 7.44 $\mathrm{mL\ d^{-1}}$ (95% CI). Back-calculated time-weighted average concentrations (TWAC) indicated that longer deployments over than 48 h yielded reliable estimates of water-column concentrations. This study demonstrates that passive samplers offer a practical, time-integrated approach for microbial monitoring in stormwater wetlands and that back-calculation of TWACs is feasible, although further validation is needed.

KEYWORDS

Urban stormwater, constructed wetland, passive sampler, microbial monitoring

1 INTRODUCTION

Stormwater is a valuable alternative water source, but its safe reuse is limited by microbial contamination, which poses significant public health risks during harvesting and reuse (Ahmed et al., 2019). Constructed wetlands have become increasingly popular as sustainable solutions for stormwater management, as they effectively reduce pollution, enhance water quality, and lower microbial risks before the water reaches natural aquatic ecosystems (Kadlec and Wallace, 2008). Yet, effective microbial monitoring of stormwater constructed wetlands is needed to evaluate the performance of such engineered systems through the understanding of microbial inflow and outflow, and to reduce public health hazards posed by urban runoff (Meng et al., 2018).

Microbial loads and their removal can significantly vary due to site-specific operational (e.g. characteristics of the catchment area, inflow water quality, and climatic conditions) and wetland design factors (e.g. retention time, vegetation type and density) (Meng et al., 2018). Therefore, selecting appropriate sampling techniques (Erickson et al., 2013), targeting microbes beyond conventional faecal indicator bacteria (FIB) (Ahmed et al., 2019), and using suitable analytical methods (Demeter et al., 2023) are key to capturing temporal variability and microbial dynamics during storm events. Grab sampling is inexpensive and simple but provides only snapshot measurements that miss the rapid variability of storm events. Composite sampling offers more representative event-averaged microbial loads (Harmel et al., 2010), yet it can still overlook short but critical peaks, especially during the first-flush phase (McCarthy et al., 2009). Autosampler-based composite sampling is costly, and manual collection is labour-intensive (Wilson et al., 2022). Passive sampling, by contrast, captures microbial contaminants over extended deployment periods and has proven effective in wastewater surveillance, although its application in stormwater monitoring remains largely unexplored (Karamati N. et al., 2024).

The overall aim of this study is to evaluate the performance of passive samplers not only for microbial detection but also for quantification, by estimating the concentration of microbes in the water column in which the samplers are deployed. This work advances the understanding of passive sampling as a practical and scalable tool to support stormwater monitoring, water quality management, and public health protection.

2 METHODOLOGY

Three sampling campaigns were conducted in July, August, and September 2024. During each campaign, torpedo-style passive samplers (Schang et al., 2021), each containing three electronegative membranes, were deployed for four days at the inflow (INs) and the outflow (OUTw)of Troups Creek wetland. Passive samplers were collected 6, 24, 48, 72, and 96 hr after deployment. Two Sigma 900 (HACH, USA) autosamplers were installed within the INs and OUTw cabinets to enable composite sample collection. Alongside passive samplers' deployment, the autosamplers were programmed to sample 250 mL every 30 minutes to fill a 1 L bottle every two hours, starting at the beginning of the 4-day campaigns. OUTw passive samplers' deployment and composite samples' collection were after 24 or 72 h of INs sampling to consider residence time of the wetland. All passive samples and composite bottles were transported to laboratory at the Department of Civil & Environmental Engineering, Monash University for processing on that day.

Total nucleic acid (TNA) was extracted using MagMax Microbiome Ultra Nucleic Acid Purification kit (Applied Biosystems, USA) and the Kingfisher Apex instrument (Thermo Fisher Scientific, USA). Quantitative polymerase chain reaction (qPCR) was used to evaluate the extracted samples. Positive and negative controls were included and quality control of qPCR followed EMMI guidelines (Borchardt et al., 2021). Limit of detection (LoD) and limit of quantification (LoQ) of the qPCR assay were also calculated using Forootan et al., (2017)'s method. Descriptive analysis was conducted to summarize the data collected. After all quality controls, qPCR results of samples were compared to calculated LoD and LoQ. R-studio v. 4.2.2 (R Core Team, 2022) was used for visualization and statistical analysis in this study

To compare the relative accumulation of CrAssphage over time on passive material, the accumulation ratios were calculated by dividing the copy numbers of CrAssphage obtained from passive samplers at each time point by the copy numbers per liter of time-weighted mean concentration of composite samples. Quantile linear regression ($\tau = 0.5$) was used to model the median accumulation ratio over time with bootstrapping of 1000 iterations to derive 95% confidence intervals around the median regression fit.

3 RESULTS AND DISCUSSION

CrAssphage detection was more consistent, both at INs and OUTw, even under lower rainfall conditions (total rainfall <5 mm), suggesting its longer environmental persistence or human inputs from upstream. CrAssphage was consistently detected in both composite and passive samplers across all time points at the INs and OUTw, even during low-rainfall event (< 5 mm) at INs samples (data not shown here). The *E. coli* concentration was measured for all composite samples at each time point, and Spearman's rank correlation analysis revealed strong positive associations between CrAssphage and *E. coli* concentrations at the INs of wetland across storm events, but this relationship diminished or reversed at the OUTw, suggesting differential removal or persistence of these indicators within the wetland system.

CrAssphage accumulation on electronegative membranes increased over time during rainfall events. The electronegative membrane proved to be an effective time-integrated sampler for CrAssphage in stormwater, with the median accumulation ratio rising from roughly 0.002 at 6 h to 0.03 at 96 h; fitting a median-quantile model (Figure 1) yielded a statistically significant linear accumulation rate of 7.44 mL d^{-1} ($p < 0.05$), confirming that the membrane remained in its integrative phase for at least four days. Prior research showed that similar electronegative membranes had linear uptake for other viral targets in wastewater i.e. PMMoV (24 mL d^{-1}), HAdV 40/41 (7.2 mL d^{-1}), and enterovirus (794.4 mL d^{-1}) (Li et al., 2022). The different uptake rate of CrAssphage in stormwater (this study) compared to enteric viruses' uptakes in wastewater (Li et al., 2022) can be related to the microbial targets' characteristics and the water sources chemistry that the passives were deployed. High TSS in wastewater may accelerate viral detection on electronegative membranes, though competing particles can also accumulate inhibitors (Hayes et al., 2022). Furthermore, pH of water sources governs how strongly like-charged viruses can overcome electrostatic repulsion and bind to electronegative membranes with viral (F-RNA bacteriophage MS2) adsorption lowest in alkaline (pH=9) conditions and increasing as the matrix becomes more acidic (pH=5) (Shakallis et al., 2024).

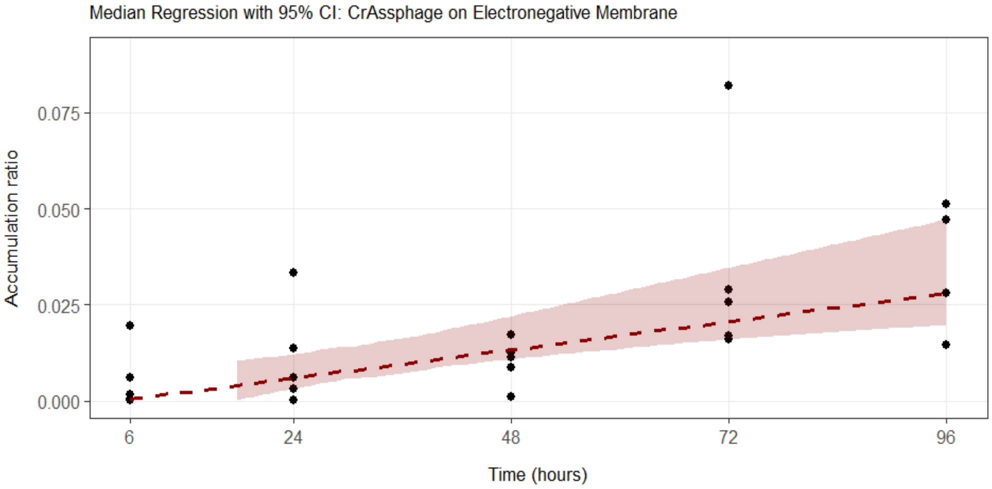

Figure 1. Median-based regression of CrAssphage accumulation ratio over time on electronegative membrane with reference to the bacteriophage concentration in composite water samples (Accumulation ratios are from three events of INs and two events of OUTw at each time point. Black dots are the median of each event's accumulation ratio at that point. The shaded area shows a 95 % confidence interval).

We back-calculated time weighted average concentrations (TWAC) of CrAssphage derived from electronegative membranes and compared it with autosampler measured TWAC (data not shown here). The results indicate that back-calculated TWACs converging towards autosampler values as deployment time increased, narrowing median differences from ~90 % at 6 h to 24% at 72 h and 39% at 96 h. These results showed that longer deployments (≥48 h) produced closer estimates to autosamplers, confirming reliable back-calculation at extended times. Discrepancies at early time points reflected low detection frequency (<75%), consistent with reports that higher viral loads improve adsorption to electronegative membranes.

4 CONCLUSION

This study demonstrates that passive electronegative membranes can reliably detect and quantify CrAssphage in stormwater-constructed wetlands when deployed for sufficient durations. Longer deployments (≥48 h) significantly enhanced detection consistency and supported accurate estimation of accumulation rates using quantile regression. The derived accumulation rate (7.44 mL day^{-1}) indicates a stable linear uptake despite variable stormwater conditions. Back-calculated TWACs closely matched water-column concentrations achieved from automatic samplers, confirming the potential for passive samplers to produce time-integrated microbial estimates. Overall, this research demonstrates that passive sampling is not only a practical and scalable monitoring approach but also a viable alternative where autosamplers are impractical or too costly to operate. By combining passive sampling with molecular technologies, this work establishes a foundation for broader application in stormwater surveillance. While further validation across additional microbial targets and sites is recommended to support broader adoption.

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