CrAssphage as an indicator of groundwater-borne pollution in coastal ecosystems

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Abstract

Novel approaches for monitoring coastal water quality changes and identifying associated contaminant source(s) are of growing importance as climate change and population redistribution to coastal zones continue to impact coastal systems. CrAssphage, a virus found in the human gut and shed with fecal matter, is currently gaining popularity as an indicator of human fecal contamination in surface water and groundwater. Here we demonstrate that DNA assays targeting crAssphage genetic fragments can be used to detect pollution from nearshore onsite wastewater treatment systems discharging to the ocean via submarine groundwater discharge. We integrated this novel viral monitoring tool into a field study that characterized the physical hydrogeology (hydraulic gradients, hydraulic conductivity, and seepage fluxes) and surface water and groundwater quality at a study site on the north shore of Nova Scotia, Canada. Increased use of onsite wastewater treatment systems during the summer cottage season coincided with widespread detections of crAssphage in submarine groundwater discharge (4/4 samples) and coastal surface waters (3/8 samples). Conversely, classical fecal pollution indicators based on bacterial targets (Escherichia coli and human-specific Bacteroidales genetic marker (HF183)) were sparsely detected in the samples in the coastal environment (2/12 E. coli samples, 0/12 HF183 samples), likely due to greater attenuation of bacterial contaminants within the subsurface environments. Results from this first application of crAssphage in coastal groundwater contribute to a growing body of research reporting the application of this emerging tracer in various environments impacted by sewage pollution sources.

1. Introduction

Coastal groundwater and surface water quality is of growing importance given recent and projected increases in coastal population density (Mallin et al 2000, Neumann et al 2015), with concomitant industrial activity and contaminant loading (e.g., Howarth 2008, Michael et al 2017). Coastal waters provide important societal and economic services for local populations and tourists alike (Völker and Kistemann 2011, DeFlorio-Barker et al 2015); however, these services are threatened by coastal pollution. For example, recreational activities in contaminated waters can lead to disease outbreak and in severe cases, death (Graciaa et al 2018). Similarly, fisheries and aquaculture operations can be impacted by the presence of pathogenic microorganisms, resulting in a loss of economic activity in the case of harvesting restrictions, or a range of negative health outcomes if products are consumed (Howarth 2008, Malham et al 2014, Vikas and Dwarakish 2015).

Coastal groundwater systems are also changing due to seawater intrusion induced by rising seas and increased coastal flooding (Werner et al 2013, Ketabchi et al 2016). In unconfined coastal aquifers, fresh groundwater ‘floats’ above the denser saline groundwater located in a salt wedge (figure 1). These freshwater zones in coastal aquifers often serve as important water supplies but are threatened by rising sea levels (Sawyer et al 2016, Michael et al 2017, McKenzie et al 2021). Changing coastal groundwater dynamics will also influence
contaminant transport processes and negatively impact potable coastal groundwater supplies and the marine ecosystems receiving groundwater discharge (LeMonte et al. 2017, Guo et al. 2020). Submarine groundwater discharge (SGD) is defined as the flow of fresh and circulated saline groundwater (e.g., LeRoux et al. 2021) from the seabed to the coastal ocean, regardless of fluid composition or driving force (Burnett et al. 2003). Coastal aquifers and associated SGD provide a conduit for chemical and biological contaminants to enter coastal surface waters (Boehm et al. 2004, Moore 2010, Sawyer et al. 2016, Robinson et al. 2018, Ruiz-González et al. 2021). One potential source of contaminants in coastal settings is Onsite Wastewater Treatment Systems (OWTS), a decentralized form of domestic wastewater management that involves the discharge of partially treated wastewater effluent into the subsurface environment. This effluent subsequently enters groundwater systems, and, in the case of coastal aquifers, can be transported to the ocean via SGD (de Sieyes et al. 2008, Izbicki et al. 2012). Coastal OWTS are particularly vulnerable to climate change and coastal storms, as elevated groundwater tables resulting from sea-level rise (SLR), high spring tides, or storm surges will impact OWTS performance (Cooper et al. 2016, Cox et al. 2019). In general, improper OWTS design can result in the introduction of untreated or partially treated wastewater effluent to groundwater systems and receiving surface water bodies (figure 1).

OWTS are a potential source of pathogenic microorganisms (e.g., bacteria, viruses, protozoa) that could be transmitted to people using coastal waters. Historically, bacteria such as Escherichia coli (E. coli) have been used as indicators of fecal pollution in both groundwater and surface water due to, among other factors, their relatively inexpensive detection methods and general occurrence across human and animal feces (Edberg et al. 2000, Health Canada 2012). Recent advances in molecular technologies, such as quantitative polymerase chain reaction (qPCR), have also led to the development of new tools for assessing sources of fecal contamination. The HF183 qPCR assay, for example, targets a Bacteroides 16S ribosomal ribonucleic acid (rRNA) gene marker, that is specific to humans and is applied to measure human fecal pollution in the environment (Haugland et al. 2010). However, there are limitations with using bacteria-based monitoring targets for assessing pathogen transport from groundwater-derived fecal contamination sources. For example, the size and shape of enteric bacteria, such as E. coli, typically leads to greater attenuation in porous media due to physical straining (figure 1, inset), resulting in effective removal of fecal bacteria within an OWTS (Stevik et al. 1999, O’Luanaigh et al. 2012). Viruses, in particular, have different transport and attenuation characteristics than bacteria, due to their small size and greater persistence in groundwater environments (Morrison et al. 2020, Pang et al. 2021). Several studies have documented the presence of enteric viruses in groundwater systems in the absence of conventional enteric bacteria indicators (Fout et al. 2017, Morrison et al. 2020, Pang et al. 2021).

Figure 1. Conceptual diagram showing an OWTS discharging effluent wastewater into groundwater, which is subsequently discharged into the ocean via SGD. Image inset shows green bacteria being strained and attenuated, while smaller and more mobile purple viruses pass through the pore space.
The recent discovery of a bacteriophage, crAssphage, that is associated with bacteria inhabiting the human intestinal tract, has provided a practical virus-based fecal indicator (Ballesté et al. 2019, Kongprajug et al. 2019, Sala-Comorera et al. 2021). First discovered in 2013, crAssphage is a novel viral marker that is host-specific and shed with human fecal material in high quantities (Dutilh et al. 2014). The specificity of the crAssphage used in this study (CPQ-056; Stachler et al. 2017) has been assessed in several geographies, with specificities ranging from 87 to 98% (Stachler et al. 2017, Ahmed et al. 2018, Gyawali et al. 2021). Cross-reactions with dog, cat, gull, and poultry fecal samples have been reported, but they are infrequent, and concentrations of the genetic marker in these samples have been orders of magnitude lower than those observed in human fecal samples. CrAssphage assays have also been shown to be highly sensitive, typically detected in 100% of raw sewage samples, with concentrations ranging from 7–9 log copies l⁻¹ (Ahmed et al. 2018, Gyawali et al. 2021). These studies have also demonstrated that concentrations are comparable to concentrations of widely used bacterial markers (e.g. HF183), and orders of magnitude higher than other enteric viruses, such as F-RNA phage and norovirus. Gyawali et al. (2021) also found that the presence of crAssphage in shellfish samples was highly predictive of norovirus contamination. Past crAssphage studies have primarily focused on pollution sources released directly to surface waters, and have largely demonstrated congruence with bacteria-based indicators (Ballesté et al. 2019, Sala-Comorera et al. 2021). Morrison et al. (2020) were the first to assess crAssphage as a monitoring tool in subsurface environments and concluded that it is a promising tool; however, they did not directly compare crAssphage to conventional bacteria-based indicators.

The study of crAssphage is an emerging research topic with only very recent applications in groundwater and surface water. However, to our knowledge, no prior studies have investigated the efficacy of crAssphage to detect human fecal pollution in SGD, or directly compared crAssphage to bacteria-based indicators in settings where the fecal pollution is primarily derived from groundwater sources. Such conditions represent the environmental settings where this monitoring tool could potentially have the most impact and value. This represents a critical knowledge gap given the establishment of SGD as a major research theme in hydrology, hydrogeology, and oceanography (Burnett et al. 2003, 2006, Moore 2010, Robinson et al. 2018, Taniguchi et al. 2019, Alorda-Kleinglass et al. 2021), with implications for coastal water quality and ecosystem health. The objectives of this study are to (1) evaluate the presence of human fecal contamination in a coastal system impacted by OWTS using crAssphage, HF183 and E. coli as fecal pollution markers and (2) compare the performance and relative sensitivities of these markers both before and during cottaging season (associated with elevated levels of fecal pollution from discharging OWTS).

2. Methods

2.1. Study site

This study was conducted at a provincial park and public beach in northern Nova Scotia, Canada, that is surrounded by private cottages (figure 2). The local climate is typical for the Canadian Maritimes with average summer (June—August) air temperatures of approximately 19 °C and mean annual precipitation of 969 mm (Government of Canada 2021, ECCC Station 8205774). Use of the beach and the surrounding private dwellings is concentrated in the summer months. The provincial park and all dwellings in the immediate area use OWTS for domestic wastewater management. The underlying geology of the site consists primarily of interbedded sandstone and siltstone units overlain by glacial tills (Hennigar 1972). Recharge to the underlying bedrock aquifer is estimated to be between 180–220 mm year⁻¹ based on the baseflow from this watershed (Kennedy et al. 2010). Groundwater monitoring and sampling took place in the surficial aquifer, which is primarily sand along the beach and sandy clay in the upland beginning approximately 20 m beyond the high tide mark. The maximum tidal range recorded by our tidal logger (figure 3) was approximately 2.50 m. The nearshore bathymetry is characterized by a shallow slope (e.g., < 5 m depth 1 km from shore).

2.2. Hydrogeology field work and data analysis

The site was instrumented in the fall of 2020 with a transect of piezometers and a tidal logger (figure 2). Piezometers were installed in the surficial aquifer using a backpack drill to depths of 2–3 m. Pressure sensors (Solinst, Canada and Onset, U.S.A.) were deployed to record pressure every 30 min in each piezometer, as well as the tidal signal just offshore (figures 2, 3). Ambient air pressure was recorded in the top of a vented provincial monitoring well located approximately 10 km from the study site.

The hydraulic diffusivity of the unconfined sand aquifer along the beach was estimated using the tidal amplitude and the resultant damped groundwater tidal response in piezometer 2 approximately 160 m from the mean water edge (equations 8 and 9 of Nielsen 1990). Hydraulic conductivity was calculated from hydraulic diffusivity by estimating the beach sand aquifer depth to be ~7 meters based on the upper end of sand thickness recorded in Nova Scotia shoreline well logs and by assuming perfect drainage of the sediment, such that specific
yield equaled sand porosity (0.25). Average linear groundwater velocity ($v$) and travel time ($t$) in the beach aquifer (which represents the groundwater flow path from the cottages located along the beach edge (figure 2)), was then estimated using the calculated hydraulic conductivity ($K$), the mean hydraulic gradient between the beach piezometer and the coast ($i$), the cross-shore beach length ($L$), and the sand porosity ($e$):

$$v = \frac{-Ki}{e} \quad \text{and} \quad t = \frac{L}{v}$$

Seepage meters (Lee et al. 1977, Duque et al. 2020) were installed approximately 200 m from the high-tide waterline, such that they were just submerged during low tide (figure S1, supplement (available online at stacks.iop.org/ERC/4/051001/mmedia)). Volumetric measurements of water collected in the seepage meter bags over recorded time intervals yielded the SGD flux of water from the seabed to the ocean. Water quality samples were collected from the seepage bags approximately every 12 h during the first field campaign in June of 2021, and approximately every 24 h during the second field campaign in August 2021 (Peeler et al. 2006).

2.3. Sampling and water quality analysis

Water samples from the seepage meters, surface water at several locations along the coastline, and streams draining into the study area (figure 2) were analyzed for chemical and microbiological parameters. Sampling was completed on single days during separate, weeklong field campaigns: the first on June 16, 2021, and the second on August 31, 2021, close to the end of the cottaging season. In both sampling campaigns, six open seawater samples were retrieved, as well as samples from the outlets of the nearby creeks (one in June and two in August). Additionally, four water quality samples were retrieved from the water collected in the seepage meter bags.

During the August campaign only, two samples were collected from the water immediately surrounding the seepage meters, to compare SGD water quality with that of open seawater in the immediate area. As radon is enriched in groundwater compared to surface water (Hoehn and Von Gunten 1989), radon analysis was completed on the sampled water using a RAD 7 (DURRIDGE Company Inc., MA, U.S.A.), and electrical conductivity readings of the collected water and surrounding seawater were recorded using a calibrated Conductivity Plus meter (Herron Instruments Inc., Dundas, ON).

Microbial parameters included E. coli, HF183, and crAssphage. Samples were collected in sterilized 1 L Nalgene collection bottles (Thermo Fisher Scientific, Waltham, MA, USA), fully submerged to 5cm depth, capped underwater, and kept on ice packs while being transported back to the laboratory at Dalhousie University in Halifax, Nova Scotia for immediate analysis. Seepage meter bags were emptied into similar 1L Nalgene collection bottles and transported in the same manner as surface water samples. For E. coli enumeration, 100 ml samples were analyzed using the membrane filtration method with mcolblue-24 selective growth media (Hach 1999). For HF183 and crAssphage markers, 500 ml sample volumes were filtered (0.45 μM pore size, 47 mm diameter, Millipore, Inc., Bedford, MA, USA), and DNA was extracted from the filters using a DNeasy PowerSoil Pro kit (Qiagen Inc., Toronto, Ontario, Canada). The concentration and purity of genomic DNA was first measured by ultraviolet absorbance spectrophotometry at 260/280 nm and 260/230 nm (Implen NanoPhotometer™, Implen, München, Germany). The qPCR assays for HF183 (Haugland et al. 2010) and crAssphage (Stachler et al. 2017) were conducted on a Bio-Rad CFX96 Touch™ Real-Time PCR detection system (Bio-Rad, Hercules, CA, USA). The limits of detection (LOD) of HF183 and crAssphage markers are 1.1 Log copies/100 ml and 2.83 Log copies/100 ml, respectively.

3. Results and discussion

3.1. Groundwater field data and analysis

The hydraulic heads obtained from the piezometers and tidal logger are presented in figure 3. The ratio of the mean tidal range in the beach piezometer (0.50 m) to the mean tidal range in the strait (2.41 m) is 0.21, which yielded an aquifer diffusivity of 2400 m$^2$/hour and a corresponding $K$ (based on storage and thickness values described earlier) of 87 m hr$^{-1}$. Based on equation (1), the tidally averaged hydraulic gradient (0.0018) between the beach piezometer and the tidal logger, and the mean cross-shore beach dimensions (200 m), an approximate travel time through the beach aquifer of 13 days was calculated. As many cottages line the beach (figure 2), this represents the travel time from the nearest OWTS to the strait.

Our seepage meters indicated an upwelling SGD flux between $2.0 \times 10^{-2}$ meters/day and $4.2 \times 10^{-3}$ meters/day, which is lower than in many previous studies (e.g., Taniguchi et al. 2002). The electrical conductivity of the water collected in the seepage meter bags indicated lower salt contents (31,881 μS cm$^{-1}$, on average) compared to the surrounding seawater (45,200 μS cm$^{-1}$), which suggests that the SGD was made up of both fresh groundwater discharge and circulated seawater. Water samples for radon analysis were taken from a seepage meter bag, as well as the surrounding seawater to test for elevated radon concentrations, a common
indicator of water with a groundwater origin (Burnett and Dulaiova 2003). The seepage meter samples had a mean radon concentration of 77 Bq m$^{-3}$, while the seawater had a lower concentration of 24.6 Bq m$^{-3}$, which supports our assumption that the water collected in the seepage meters is in part derived from the terrestrial aquifer and thus could be impacted by nearby OWTS.

Figure 2. Map of the study site in Nova Scotia, Canada, with residential properties indicated by grey squares. East and West streams (blue lines) referenced in table 1 can be seen in the bottom left (West) and middle (East, within the public park) of the map.

Figure 3. Groundwater (top, piezometer locations indicated in figure 2) and surface water (bottom, strait) head data from the study site during the study period.
Table 1. Results from the water quality sampling during both the June and August sampling campaigns (see figure 2 for locations). Bolded values indicate microbial targets exceeding detection limits.

|                | E. coli (CFU/100 ml) | HF183 marker (Log copies/100 ml) | crAssphage marker (Log copies/100 ml) |
|----------------|----------------------|----------------------------------|--------------------------------------|
| June 2021      |                      |                                  |                                      |
| Beach A1 a     | <1                   | <1.1                             | <2.83                                |
| Beach A2       | 1                    | <1.1                             | <2.83                                |
| Beach A3       | <1                   | <1.1                             | <2.83                                |
| Beach A4       | <1                   | <1.1                             | <2.83                                |
| Beach A5       | <1                   | <1.1                             | <2.83                                |
| Beach A6       | <1                   | <1.1                             | <2.83                                |
| West Stream    | 240                  | <1.1                             | <2.83                                |
| Seepage A1 b   | <1                   | <1.1                             | <2.83                                |
| Seepage A2     | <1                   | <1.1                             | <2.83                                |
| Seepage A3     | <1                   | <1.1                             | <2.83                                |
| Seepage A4     | <1                   | <1.1                             | <2.83                                |
|                |                      |                                  |                                      |
| August 2021    |                      |                                  |                                      |
| Beach B1       | <1                   | <1.1                             | 2.85                                 |
| Beach B2       | 3                    | <1.1                             | 2.86                                 |
| Beach B3       | <1                   | <1.1                             | 2.83                                 |
| Beach B4       | 6                    | <1.1                             | 2.85                                 |
| Beach B5       | <1                   | <1.1                             | 2.83                                 |
| Beach B6       | <1                   | <1.1                             | 2.83                                 |
| Seepage B1     | <1                   | <1.1                             | 2.85                                 |
| Seepage B2     | <1                   | <1.1                             | 3.78                                 |
| Seepage B3     | <1                   | <1.1                             | 3.75                                 |
| Seepage B4     | <1                   | <1.1                             | 2.89                                 |
| Seawater 1 c   | <1                   | <1.1                             | 2.83                                 |
| Seawater 2     | <1                   | <1.1                             | 2.83                                 |
| East           | 8                    | 1.28                             | 3.05                                 |
| Stream d       | 11                   | 1.97                             | 2.98                                 |

a ‘Beach’ samples reference open seawater,
b ‘Seepage’ samples were drawn directly from the water collected in the seepage meter bags.
c ‘Seawater’ samples 1 and 2 were drawn from seawater directly beside the seepage meters.
d East and West streams can be seen on the map (figure 2).

3.2. Coastal water quality analysis

Results of both the June and August sampling campaigns for the three fecal indicators (E. coli, HF183, and CrAssphage) are presented in table 1. E. coli was absent during the June sampling campaign, except for the West Stream and a single seepage meter in very low quantities (1 CFU/100 ml), while HF183 and CrAssphage were not detected. The elevated presence of E. coli in the creek could be the result of upstream agricultural practices, including observed livestock farming. The August sampling event revealed elevated E. coli levels in two of the four seepage meters, as well as presence in both the East and West streams, albeit in lower quantities compared to June. HF183 was absent in all sampling events, excluding the August sampling of the two streams.

The absence of both E. coli (2/11 detections in June, 4/14 detections in August) and HF183 (0/11 detections in June, 2/14 detections in August) markers in the majority of the samples in the coastal waters and streams (table 1) is likely the result of the porous media successfully filtering these contaminants via straining and adsorption, as has been demonstrated for bacteria in many column experiments and field investigations (e.g., Bradford et al 2006, Jiang et al 2007). CrAssphage was undetected during the June sampling event compared to its detection in nine of fourteen samples during August sampling (table 1). CrAssphage was present in three of six open seawater samples (maximum concentration of 2.86 log copies/100 ml), and in all four seepage meter samples (maximum concentration of 3.78 log copies/100 ml), suggesting that groundwater was a source of this enteric virus. The presence of crAssphage in August, particularly in the groundwater (seepage samples), compared to the complete absence in June (table 1) suggests that increased OWTS usage during the summer contributes to wastewater loading to the shoreline via SGD pathways. Septic systems in this area consist of a septic tank that stores waste for 3–4 days, before discharge to a soil absorption field. Effluent discharge would be limited to the summer months when the septic system is being loaded. The ∼13 day groundwater travel time...
(section 3.1) is short enough to result in different coastal water quality conditions before and after cottage season based on SGD-borne contamination from OWTS. This indicates that contamination could occur seasonally, with a spike each year during cottage season when the OWTS see increased usage.

3.3. CrAssphage as a monitoring tool

OWTS are used widely for domestic wastewater treatment in rural settings, many of which are also coastal. In the United States, more than one in five homes utilize OWTS for their wastewater treatment (EPA 2013). In Nova Scotia, OWTS are used by nearly half of the province’s 1 million residents (Nova Scotia Environment 2011). Many of these OWTS are coastal and potentially at risk of subsurface inundation due to sea-level rise (James et al 2015). Globally, coastlines are widely used for recreation as well as aquaculture, both of which are sensitive to contaminant loading from SGD (Ghermandi and Nunes 2013). In this study, we demonstrate the efficacy of crAssphage as a monitoring tool for fecal contamination from diffuse, groundwater-derived pollution sources. Early detection of human fecal contaminants in coastal waters enables preemptive action in identifying sources and initiating pollution mitigation programs.

4. Summary and conclusion

Our findings add to a growing body of literature highlighting crAssphage as a useful indicator of fecal pollution. Specifically, we demonstrate its efficacy for studying coastal pollution from SGD. We highlight the ability of crAssphage to detect fecal pollution from OWTS in a coastal setting, while demonstrating the impacts of seasonal contaminant loading, likely resulting from increased use of cottages reliant on OWTS for wastewater disposal. While fecal contamination at many coastal sites may be low today, enhanced rates of SLR will impact OWTS performance and lead to elevated contaminant levels in the future. Further studies could attempt to relate crAssphage concentrations to the presence and levels of other pathogenic viruses and related human health outcomes. We suggest that crAssphage could be an important tool for detecting viral contamination of coastal recreational waters impacted by OWTS.

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Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

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