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1   **Fate of antibiotic resistance genes in two Arctic tundra wetlands impacted by municipal**
2   **wastewater.**

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14   **Highlights**

- 15   • Antibiotic resistance genes (ARGs) were assessed in two tundra wastewater wetlands.
- 16   • Hydrology of the wetlands was an important factor for ARG concentrations.
- 17   • Spring runoff increased ARG concentrations in water with high flows.
- 18   • Un-impacted reference wetlands were assessed for ARGs.
- 19   • Preliminary first order rate constants were ARG specific.

20

Abstract

In the Canadian Arctic, it is common practice to discharge municipal wastewater into tundra wetlands. Antibiotic resistant bacteria and the antibiotic resistance genes (ARGs) they contain can be present in municipal wastewater and there is a scarcity of knowledge on ARGs in wastewater in Arctic environments. This study was initiated on the fate of ARGs in tundra wetland ecosystems impacted by anthropogenic wastewater sources in Arctic communities. In the summer season of 2016, two wetlands were studied in the Inuit communities of Sanikiluaq and Naujaat in Nunavut, Canada. Genomic DNA was extracted from both soil and water during the spring freshet and late summer in the wetlands, and a suite of nine clinically relevant ARGs (*sul1*, *sul2*, *mecA*, *vanA*, *qnrS*, *ermB*, *tetO*, *bla_{TEM}*, *bla_{CTX-M}*), and an integron gene (*int1*) were analyzed using quantitative polymerase chain reaction (qPCR). Hydrological and water quality measurements were conducted in conjunction with the microbiological sampling. Gene targets were consistently present in the wastewater, and throughout both wetlands, except for *vanA* and *mecA*. Concentrations of ARGs were greater during the spring freshet, due to short hydraulic retention times (< 2 days), which coincided with decreased treatment performance. The natural resistome in un-impacted wetlands had above detection limit concentrations of *int1*, *sul1*, *sul2*, *bla_{CTX-M}* in water in Naujaat, and *sul1*, *qnrS* and *tetO* in soil in Sanikiluaq. First-order rate constants were widely variable and specific to the gene target. ARGs were present in concentrations elevated above baseline reference sites in tundra wetlands influenced by municipal wastewater, and hydrological conditions had a large impact on their spatial distribution and levels.

Keywords: ARG, tundra wetland hydrology, qPCR, cold climate, Arctic, antimicrobial resistance

1. Introduction

The development of antimicrobial resistance (AMR) has become a prevalent global public health issue (Davies & Davies, 2010). Antibiotics are present in municipal wastewater, originating from partially metabolized medications used by humans and disposal of un-used antibiotics (Nagulapally et al., 2009). Antibiotic resistant bacteria (ARB), and associated antibiotic resistance genes (ARGs), can accumulate and persist in human and agricultural wastes and then be applied or released into terrestrial and aquatic environments (Ashbolt, 2013; Czekalski et al., 2012). These AMR contaminants pose human health risks as previously curable infections are now becoming resistant to conventional antibiotics (Ashbolt, 2013; Laxminarayan et al., 2013). Research has begun to demonstrate that some environments can behave as reactors for ARB proliferation, such as wastewater treatment plants (Czekalski et al., 2012; Rizzo et al., 2013). Soil and water environments can function as environmental reservoirs for ARGs (Martinez, 2008; Taylor et al., 2011), and it has been demonstrated that some ARGs are part of the ancient soil microbiome (D'Costa et al., 2011). Potential for convergence of ARGs between environmental soil bacteria and human pathogens has been suggested by Forsberg et al. (2012). In some regions the baseline environmental levels of ARGs have been observed to be increasing under anthropogenic pressures (Knapp et al., 2009). There is concern that increasing anthropogenic use of antibiotics is contributing to selective pressure and increased risk of horizontal gene transfer to human pathogens (Qiu et al., 2012). Characterization of the persistence of ARGs is crucial for human health risk assessment (HHRA) (Bouki et al., 2013). Specifically, quantification of the clinically relevant AMR bacteria within the potential exposure sites to humans and animal vectors within the environment is required to inform quantitative microbial risk assessment (QMRA), and this type of data has been reported to be limited

(Huijbers et al., 2015). One knowledge gap in the HHRA process includes quantification of ARGs and AMR bacteria hot spots in the environmental dimension, specifically soil and aquatic systems (Ashbolt et al., 2013).

The extreme climate and remoteness of the expansive region of the Canadian Arctic restricts wastewater management options, increases costs and often leads to difficulties with operation and maintenance of wastewater infrastructure (Johnson et al., 2014; Yates et al., 2012). The majority of communities in Canada's Far North use centralized methods of wastewater management with passive treatment systems comprised of lagoons (Johnson, 2008); also referred to as wastewater stabilization ponds (WSPs). In many communities, these systems are not engineered, and consist of a natural depression in the landscape. Due to permafrost, most communities have trucked water distribution systems for drinking and wastewater with storage on an individual household level (Daley et al., 2014). Engineered lagoons operate as one-year detention controlled discharge storage ponds which remain frozen most of the year. Typically, discharge occurs at the end of a three-month "treatment season" spanning from late-June to early-September (Ragush et al., 2015). Conversely, non-engineered systems release wastewater in an uncontrolled manner at the start of the spring thaw or freshet, continuing throughout the summer. The lagoons often discharge to tundra wetlands, which are natural features of the landscape where polishing of effluent has been observed prior to discharge into primarily marine receiving water environments (Yates et al., 2012; Hayward et al., 2014; Balch et al., 2018). These wetland areas have been used for wastewater disposal for decades (Balch et al., 2018), and have been termed Wetland Treatment Areas (WTAs). The treatment performance and attenuation of conventional wastewater contaminants within tundra WTAs has been observed to have high inter- and intra-system variability governed largely by the natural hydrology of the landscape and

temperature with increased treatment observed when hydraulic retention times (HRTs) are sufficient to allow treatment (Hayward et al., 2014; Balch et al., 2018). For example, Yates et al. (2012) reported WTAs were effective for wastewater treatment with contaminant removal rates in six WTAs over an Arctic summer which ranged from 47 – 94% for five-day biochemical oxygen demand (CBOD₅), 39 – 98% for total suspended solids (TSS), >99% for *Escherichia coli* (*E. coli*), 84 – 99% for un-ionized ammonia (NH₃-N), and 80 – 99% for total phosphorus (TP).

It has been observed that the removal of human pathogens from some lagoons in the Canadian Arctic may be inadequate (Huang et al., 2017). Due to lack of disinfection, there may be an elevated risk of development of AMR in the microbial communities associated with Arctic wastewater treatment systems (WWTSs), which is exacerbated by low ambient temperatures and low biological diversity in the receiving waters (Gunnarsdóttir et al., 2013). A potential contributing problem in Arctic environments includes the demonstrated potential for longer survival time of some bacteria in cold temperatures (Howell et al., 1996). Although population sizes in the Canadian Arctic are relatively small—with few commercial agriculture and aquaculture industries—there is still cases of higher reported incidence of enteric infection in comparison to southern Canada (Harper et al., 2015) and AMR could be problematic (Gunnarsdóttir et al., 2013). Since WWTSs are located within or near many northern and remote communities, human-environment interactions could pose a risk of exposure to pathogens associated with wastewater via direct contact with the landscape or aquatic environment, or through contact with wildlife vectors (Daley et al., 2015; Pardhan-Ali et al., 2013; Founou et al., 2016; Harper et al., 2011). The possibility for wildlife to act as vectors for spreading of AMR from urban to rural areas in Arctic environments has been demonstrated in Alaskan seagulls carrying antibiotic resistant strains of *E. coli* (Atterby et al., 2016; Ramey et al., 2017).

However, there have been limited studies conducted specifically on AMR in the Arctic due to the logistical and financial constraints associated with travel to these remote communities. Chaves-Barquero et al. (2016) conducted a study in Cambridge Bay, Nunavut, Canada to assess the concentrations of pharmaceuticals and ARGs (conferring resistance to tetracycline and sulfonamide) in the effluent from a wastewater lagoon and downstream tundra wetland. They concluded that ARGs were found in the lagoon and wetland system and were largely diluted in the marine receiving waters. Neudorf et al. (2017) studied three WWTSS in Nunavut, Canada including two lagoon systems (Pond Inlet and Clyde River), and one mechanical treatment plant (Iqaluit). Their study demonstrated that ARGs (2 log gene copies per mL) were present in the effluent discharged into the receiving environments, which could pose a risk for horizontal gene transfer to pathogens. The actual risk posed to human health by ARGs in Arctic receiving environments is unknown at this point. To address this knowledge gap, Neudorf et al. (2017) stated that further research into ARG prevalence and behaviour in Arctic environments is warranted.

It is hypothesized that the ARG concentrations in the WTAs will follow trends observed in previous conventional wastewater quality studies on tundra wetlands which showed improvements in water quality as the treatment season progressed (Yates et al., 2012; Hayward et al., 2014; Balch et al., 2018). In addition, it is hypothesized that the geographic location of the wetlands may contribute to the observed treatment, with higher latitudes having potentially less precipitation leading to decreased dilution, and lower temperatures, which can slow biological treatment. The overall objective of this study was to quantify the levels of nine ARG targets in soil and water within two tundra wetlands impacted by municipal wastewater in the northern territory of Nunavut, Canada. This study specifically examined the: (i) seasonal hydrologic

variability effects on the spatial distribution and levels of ARGs with the two treatment wetlands,
(ii) natural resistome of un-impacted reference wetlands, and (iii) kinetics of ARG removal.

2. Methodology

2.1. Site descriptions

The two study sites were located in Sanikiluaq (56°32'34" N, 079°13'30" W) and Naujaat (66°31'19"N, 086°14'16"W) in Nunavut, Canada (Figure 1). Sanikiluaq is in the subarctic region of Canada which was selected as a low latitude site with slightly higher precipitation and average summer air temperatures. Naujaat is located close to the boundary of the Arctic Circle and was selected as representative of a higher latitude site with less precipitation and lower average summer air temperatures. The populations are 882 and 1,082 for Sanikiluaq and Naujaat, respectively (Statistics Canada, 2017a & b). Average air temperatures in Sanikiluaq range from –28°C to –19°C in January, and from 6°C to 16°C in July. Total precipitation averages 671 mm, with 422 mm as rainfall, and 2,488 mm as snow (249 mm Snow Water Equivalent), (Government of Canada, 2016a). In Naujaat, average air temperatures range from –34°C to –28°C in January, and from 4°C to 13°C in July. Total precipitation averages 339 mm, with 124 mm as rainfall, and 2,154 mm as snow (215 mm Snow Water Equivalent), (Government of Canada, 2016b).

Figure 1. Map of study sites in Nunavut, Canada.

Both communities use trucked drinking water distribution and waste collection services. Approximately 88 to 100 m³/day of wastewater is collected in each of the hamlets (Hamlet of Sanikiluaq, 2015; Hamlet of Repulse Bay, 2015). In each of the communities, wastewater is deposited into lagoons, which consist of natural depressions in the landscape. In Sanikiluaq one side of the lagoon has an engineered berm to retain the wastewater. Both WTAs remain frozen throughout the winter months with freeze-up occurring in early to late-September and thaw occurring in late-May in Sanikiluaq and mid-June in Naujaat. Permafrost was not encountered at

162 depths of up to 1.5 m in Sanikiluaq, and the depth of the active layer in Naujaat was generally
163 greater than 0.3 m in the wetland. Effluent from the lagoons flows by gravity throughout the
164 treatment season into the down-gradient WTAs as shown in Figures 2 and 3. The areas and
165 lengths of the study wetlands are approximately: 3.4 ha and 1 km (Sanikiluaq); and 3.7 ha and
166 1.2 km (Naujaat). The vegetation in the study wetlands are characterized by various willows,
167 sedges, fireweed, mosses, and grasses; the upland areas were characterized by berries, white
168 heather, lichens, grasses, and mountain avens (the latter only in Naujaat). The watershed areas
169 contributing external hydrologic inputs into the wetlands were 170 ha and 96 ha, for Sanikiluaq
170 and Naujaat, respectively (Figures 2 and 3). Ultimately, the wetlands discharge effluent into
171 marine receiving environments. Data collection was conducted from May 20th – 31st and
172 September 1st – 9th, 2016 in Sanikiluaq, and from June 10th – 21st and August 24th – 31st, 2016 in
173 Naujaat. The May and June trips were representative of the spring period, when snow and ice
174 melt is occurring; while the August and September trips were representative of the summer
175 period, which is characteristically more arid in many tundra wetlands (Hayward et al., 2014).

Figure 2. a) Satellite image overview of Sanikiluaq and the WWTS showing the watershed boundary, and b) grayscale plan view map of the WTA showing the location of the sample points.

Figure 3. a) Satellite image overview of Naujaat and the WWTS showing the watershed boundary, and b) grayscale plan view map of the WTA showing the location of the sample points.

2.2. Reference wetlands

A reference wetland with similar physical attributes to the WTAs was selected at each study site. This allowed for comparison of ARG levels and general water quality between the study

wetlands and the natural tundra landscape. The locations of the reference wetlands are shown in Figures 2 and 3. Both reference wetlands were 70 m – 90 m upgradient of the intertidal zone and not under the influence of tidal action. Water quality and soil samples were collected at two locations in flowing water channels separated by 75 m in Sanikiluaq and 15 m in Naujaat within each of the reference wetlands. The sample sites were selected to capture running surface water flow from permanent drainage channels in the reference wetlands. The reference wetland in Sanikiluaq was sampled for surface water twice during each site visit. Whereas, in the reference wetland in Naujaat was sampled for surface water twice in June and only once in August. Therefore, six and five reference surface water samples were collected over the treatment season in Sanikiluaq and Naujaat respectively.

2.3. Hydraulic and hydrology characterization

Instantaneous discharge was measured within the study wetlands at the inlets, mid-points, and outlets with a 625DF2N digital pygmy meter (Gurley Precision Instruments, Troy, New York, United States). The velocity-area method was used to determine the instantaneous discharge according to Dingman (2002). Stage-discharge relationships were developed at the outlets of the wetlands and combined with continuous water level measurements collected with HOBO U20 Water Level loggers (Onset Computer Corporation, Bourne, Massachusetts, United States) to continuously measure flow. Discrete measurements of flow were conducted at the inlets. Rhodamine WT (RWT) fluorescent dye with a standard concentration of 200 g/L RWT was used to conduct tracer tests within the wetlands during each site visit to aid with wetland delineation and to characterize the hydraulic parameters of the wetlands including hydraulic retention times (HRTs) and mixing and dispersion behaviour. The tracer concentration response curves were processed according to the procedure detailed in Hayward et al. (2014). The watersheds of each

of the wetlands were delineated with ArcGIS ArcMap 10 software (ESRI, Redlands, California, United States). Digital Elevation Models (DEMs) with a spatial resolution of 30 m were sourced for each site from the Natural Resources Canada online database GeoGratis (Government of Canada, 2017). A real-time kinematic (RTK) topographic survey was conducted in Sanikiluaq due to the requirement for a finer spatial resolution DEM. The hydraulic loading rates were calculated by dividing the minimum, maximum, and average wetland discrete inflows (m^3/d) over the treatment season by the delineated wetland areas (m^2) and conversion of units to centimetres per day.

2.4. Water quality sampling and analysis

Raw wastewater samples were collected from the pump trucks to characterize untreated wastewater quality parameters and ARG concentrations. Water samples were collected from the inlet, mid-points, and outlet of the wetlands in sterilized 1L plastic sample bottles. Water samples were also collected from two locations in each of the reference wetlands. General water quality indicators (WQIs) of temperature, dissolved oxygen (DO), specific conductance, and pH were made *in situ* for each sample collection with a YSI600 handheld water quality sonde (YSI Inc., Yellow Springs, Ohio, United States), which has lower operational limits of -5°C . The sonde was calibrated according to manufacturer's specifications prior to each site visit for all parameters and daily for DO. Water samples for standard water quality parameters were stored chilled at 4°C and transported by aircraft to be analyzed within hold times at an accredited commercial laboratory.

Water samples were analyzed for CBOD₅, TSS, volatile suspended solids (VSS), total coliform (TC), *E. coli*, total nitrogen (TN), total ammonia nitrogen (TAN), $\text{NH}_3\text{-N}$, and TP according to standard methods (APHA, 2012). Quantification of a standard suite of 32 metals was analyzed

for all water samples with inductively coupled-mass spectrometry (ICP-MS). Water samples collected in June from Naujaat were analyzed at the commercial laboratory Taiga Environmental in Yellowknife, Northwest Territories. Water samples collected from Sanikiluaq in May were analyzed at the commercial laboratory Maxxam Analytics in Montreal, Quebec. All other samples were analyzed at the commercial laboratory Maxxam Analytics in Winnipeg, Manitoba. The trace metals ICP-MS scan from Taiga Environmental Laboratory was conducted according to EPA method 200.8 (USEPA, 1994). The trace metals ICP-MS scan from Maxxam Analytics Laboratory in Montreal was conducted according to method MA.200 -Mét 1.2 (Government of Québec, 2014). The trace metals ICP-MS scan from Maxxam Analytics Laboratory in Winnipeg was conducted according to Method 6020A R1 (USEPA, 1998). A total of two rounds of water samples were collected from the wetlands per each site visit (e.g., spring and summer). Water samples for general water quality and gene target analysis were collected on May 25th and May 30th, 2016, and September 6th and 8th, 2016 in Sanikiluaq. Water samples for general water quality and gene target analysis were collected in Naujaat on June 16th and June 21st, 2016, and on August 29th and August 31st, 2016 (except reference samples). A total of 1L of raw wastewater sample was collected to facilitate the general water quality and gene target analysis per each pump truck sampled. In Sanikiluaq, discrete samples were collected from one truck on May 25th, two separate trucks on May 30th, and three separate trucks on September 9th, 2016. In Naujaat, discrete samples were collected from three separate trucks on June 16th, 2016 and three separate trucks on August 31st, 2016.

2.5. Sampling and analysis of soil and water for gene targets

Soil samples were collected from the inlet, mid-point, and outlet of each wetland and at three locations within each of the reference wetlands. Soil samples were only collected once at each

site near the end of summer due to logistical constraints. Soil samples were collected on September 8th, 2016 and August 29th, 2016, in Sanikiluaq and Naujaat, respectively. Each soil sample consisted of a composite sample of three sub-samples from each sample collection point. The soil samples were collected by cutting soil with a sterilized knife from the top 0 – 10 cm of the bottom substrate and banks of the flow paths of effluent within the study wetlands and reference wetlands. Soil samples were collected directly (either submerged or partially) in the flow paths of the effluent in the WTAs. The soil samples were kept chilled at 4°C following collection.

Approximately 30 – 500 mL of each water sample was filtered through a 0.45 µm pore size filter using a Millipore Vacuum Manifold and Microfil Filtration funnels (Millipore, Inc., Bedford, Massachusetts, United States). After filtration, the filter membrane was placed in a 15 mL falcon tube with 1mL of sterilized water to prevent dry out and immediately frozen. The soil (stored at 4°C) and filters (stored frozen) were transported by aircraft to Dalhousie University in Halifax, Nova Scotia, Canada.

MoBio Powersoil DNA Extraction Kits (VWR International, Ville Mont-Royal, Québec, Canada) were used to extract the genomic DNA from the bacteria within the soil and water samples. ARG target copy numbers were detected using quantitative real-time PCR (qPCR). This study was limited to only nine of the following gene targets which were quantified within the water and soil samples for this study: class I integrase gene (*int1*), sulfonamide resistance genes (*sul1* and *sul2*), methicillin resistance gene (*mecA*), vancomycin type A resistance gene (*vanA*), fluoroquinolone resistance gene (*qnrS*), macrolide-lincosamide-streptogramin type B resistance gene (*ermB*), tetracycline resistance gene (*tetO*), and class A β-lactamase genes (*bla_{TEM}* and *bla_{CTX-M}*). The *int1* gene was analyzed because it is a genetic indicator of anthropogenic pollution

279 and is commonly associated with genes which confer resistance to antibiotics (Gillings et al.,
280 2015). A limitation to this study is that the antibiotic concentrations within the wastewater were
281 not quantified. According to the Canadian AMR surveillance system report, commonly
282 prescribed antibiotics in the northern territories include amoxicillin, azithromycin, ciprofloxacin,
283 doxycycline, and sulfamethoxazole (Government of Canada, 2016c). Of these prescribed
284 antibiotics, the following genes can confer resistance: *bla_{TEM}*, *bla_{CTX-M}* and *mecA* (amoxicillin);
285 *ermB* (azithromycin); *qnrS* (ciprofloxacin); *tet* (doxycycline); and *sul1* and *sul2*
286 (sulfamethoxazole) respectively (McConnell, 2017). The rationale for the nine gene target panel
287 was partly to characterize the genes that confer resistance to the commonly clinically prescribed
288 antibiotics as identified in Government of Canada (2016c). This suite of gene targets was also
289 selected in attempt to characterize genes which confer resistance to a range of antibiotic classes
290 which have been previously detected within a municipal wastewater treatment plant by
291 Szczepanowski et al. (2009). It should be noted that the nine gene targets are not an exhaustive
292 nor comprehensive list of the genes which confer resistance to conventional antibiotics and this
293 is a limitation of the study. The gene target suite was quantified using TaqMan qPCR on a Bio-
294 Rad CFX96 Touch system (Bio-Rad, Hercules, California, United States). The 16S ribosomal
295 ribonucleic acid (rRNA) gene copies, which were determined to enable the calculation of the
296 relative abundance of ARGs in the bacterial community, were quantified using SYBR Green
297 qPCR. A complete description of the qPCR methodologies is provided in Neudorf et al. (2017)
298 and the primer and hydrolysis TaqMan probe sequences and cycling conditions are provided in
299 the supplemental information (Table A.1). The limit of quantification (LOQ) (copies/reaction)
300 for each of the gene targets in the suite were: *int1* = 14, *mecA* = 69, *vanA* = 138, *sul1* = 12, *sul2* =
301 10, *qnrS* = 112, *ermB* = 14, *tetO* = 70, *bla_{TEM}* = 243, and *bla_{CTX-M}* = 6, and 16S rRNA = 67,000.

The limit of detection (LOD) was 5 copies/reaction (or 1 copy/mL for 500 mL sample volumes and 10 copies/mL for 50 mL sample volumes) determined according to McConnell (2017). The units of measurement for absolute abundance of gene target concentrations were determined in gene copies per mL of water or gram of sediment and presented as log transformed values.

2.6. Data analysis

The relative abundance for each gene target, except for 16S rRNA, was calculated by dividing each gene target concentration at each sample location per each sampling event by the 16S rRNA concentration and by log transforming this result, which represented log (gene copies/16S rRNA genes). The relative abundance of the gene targets characterizes the proportion of the ARG target copy concentration in the total bacterial population represented by the 16S rRNA gene. The distribution of gene targets refers to the spread of individual absolute gene target concentrations, except for 16S rRNA, at each sample point within each of the two sites.

2.7. First-order rate constant determination

The first-order removal rate constants (k) are commonly used parameters in constructed treatment wetland design to describe the rates at which conventional wastewater contaminants are attenuated in wetlands. Numerous applications of this chemical reactor contaminant attenuation theory are summarized in Kadlec and Wallace (2009). Generally, the higher the value of k , the faster the rate of removal of that contaminant from the wetland (Hayward, 2013). The first-order rate constants for the gene targets were determined with a modified tanks-in-series (TIS) chemical reactor model parameterized with site-specific data according to the procedure detailed in Hayward and Jamieson (2015). The number of TIS determined from the dye tracer data were used to construct and parameterize the Microsoft Excel spreadsheet models which were used to determine the first-order rate constants. An example spreadsheet calculator template

used to calculate the first-order rate constants is presented in CWRS (2016). The areal first-order rate constants in units of metres per year (m/y) were adjusted to 20°C (k_{20}) according to the Arrhenius temperature correction equation and coefficients described in Hayward and Jamieson (2015). The temperature adjustment of k to 20°C is standard design practice for first-order rate constants to enable comparison to other treatment wetlands. The temperature correction coefficient of 1.07 was selected based on the assumption of doubling the rate of bacterial loss for a 10°C temperature rise (Chapra, 1997; Boutilier et al., 2009). Rate constants were determined only in cases when influent and effluent ARG concentrations from the wetlands were above LOQs. There were multiple model runs per site but solely the minimum k_{20} 's are presented in the results. All k_{20} 's were calculated from log transformed input gene concentrations.

2.8. Statistical analysis

A principal components analysis (PCA) was conducted on the entire dataset (both wetlands combined) with the following parameters: *int1*, *mecA*, *sul1*, *sul2*, *tetO*, *blaTEM*, 16S rRNA, CBOD₅, TSS, VSS, TC, *E. coli*, TN, TAN, TP, WQIs, and eleven metals (aluminum (Al), barium (Ba), copper (Cu), iron (Fe), manganese (Mn), strontium (Sr), zinc (Zn), calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na)). The PCA data was log-transformed and analyzed as a correlation matrix in SigmaPlot version 13.0 statistical software. For the PCA, the samples were un-pooled, except raw samples which were pooled for each site over the entire sample period, and the reference samples which were pooled for samples collected during the same season (e.g., spring and summer). Paired student t-tests (two-tailed) were used to assess whether the raw wastewater samples, and effluent and reference samples were significantly different with significance attributed at $P < 0.05$. In all instances, S.D. is an abbreviated form of standard deviation.

3. Results and Discussion

3.1. Hydraulics and hydrology

The hydrology of both wetlands was strongly influenced by the seasonal changes in climate. High spring freshet flows occurred in late May to June, as the snow and ice which accumulated over the winter melted, with flows decreasing as the summer progressed into July and August (Figures 4a and 4b). This finding is consistent with observations of seasonal hydrological changes in the WTA studied by Hayward et al. (2014). The surface flows at the wetland inlet in Sanikiluaq were generally much lower than the flows at the outlet throughout the study period (Figure 4a), with a maximum of 529 (n = 11 days discrete) and 4,612 m³/d (n = 133 days continuous) for influent and effluent, respectively. A subsurface flow (i.e., seepage) area of approximately 1.3 ha was observed just downstream of the inlet, which in some locations had a depth of greater than 1.5 m. The subsurface flow was not quantified but likely accounted for some of the influent flow in Sanikiluaq. Average flow rates for Sanikiluaq over the study period were 196 m³/d (n = 11 days discrete) and 290 m³/d (n = 133 days continuous) for influent and effluent, respectively. Dilution was observed to range from 392 to 1,111% during the spring freshet study period in Sanikiluaq, which led to generally better water quality within the wetland and at the outlet. The hydraulic loading rates (HLRs) for Sanikiluaq ranged from 0 to 1.6 cm/d, with an average of 0.6 cm/d (n = 11 days discrete). These HLRs are relatively low, and well below typical engineering design criteria for HLRs for free water surface (FWS) wetlands, which range from 2.5 to 12.5 cm/d (Water Environment Federation, 2010). However, the HRT in Sanikiluaq during the spring freshet was determined to be 1.4 days from the dye tracer test, which is much shorter than the optimal 14 to 20 days for natural treatment wetlands (Alberta Environment, 2000; Kadlec and Knight, 1996). This may have been indicative of short-circuiting

of effluent through the wetland. During the summer period, the HRT was longer (> 7 days) due to the subsurface flow area in Sanikiluaq and negligible inflow at the inlet. The number of TIS during the spring freshet for the entire wetland was determined to be high for FWS wetlands at 19 TIS; comparatively, for context Kadlec and Wallace (2009) reported a much lower average TIS of close to 4.1 ± 0.4 S.D. based on data from 35 constructed FWS wetlands. As the number of TIS approaches infinity, the hydraulic behavior of the wetland approaches plug flow with limited internal mixing (Kadlec and Wallace, 2009), which is not ideal from a treatment perspective. During the September trip, the wetland in Sanikiluaq behaved as a plug-flow reactor. This suggests that during both seasonal periods in Sanikiluaq there was considerable short-circuiting which is not conducive to treatment processes.

The hydrology of the Naujaat wetland system possessed some differences when compared to Sanikiluaq (Figure 4b). During extended periods, the outflow was measured to be lower than the inflow, with minimum flows of $305 \text{ m}^3/\text{d}$ ($n = 13$ days discrete) and $0 \text{ m}^3/\text{d}$ ($n = 79$ days continuous), and average flows of $3,107 \text{ m}^3/\text{d}$ ($n = 13$ days discrete) and $1,117 \text{ m}^3/\text{d}$ ($n = 79$ days) at the inlet and outlet, respectively. This water deficit across the wetland may suggest that the two large ponds downstream of the mid-point in the Naujaat wetland likely acted as detention ponds, and evapotranspiration and seepage may have played a role in removal of surface water from the wetland. In Naujaat, maximum flows of $7,057$ ($n = 13$ days discrete) and $6,004 \text{ m}^3/\text{d}$ ($n = 79$ days continuous) were observed at the inlet and outlet, respectively. The HLRs over the treatment in season in Naujaat ranged from 0.8 to 17.5 cm/d , with an average of 8.5 cm/d ($n = 13$ days discrete), respectively. These HLRs were within the recommended for treatment wetlands for average but not high flow values. The results of the dye tracer tests demonstrated that similar to what was observed in the Sanikiluaq wetland, the HRT measured during the spring freshet was

394 short (19.5 hours). The HRT in the wetland improved to 8 days later in the summer. The number
395 of TIS determined within the wetland in Naujaat varied over the treatment season, with 17 TIS
396 representative of the entire wetland during the spring, and 13 TIS representative of the system
397 during August. These numbers suggest that there is plug flow-like behavior in the Naujaat
398 wetland as well.

Figure 4. Hydrographs from a) the Sanikiluaq wetland; and b) the Naujaat wetland over the treatment season in 2016. The black dots represent the discrete influent measurements collected during the site visits.

3.2. General Water Quality

The sites both met their respective water licence requirements during the study period at their outlets, except for Naujaat for bacteria in the spring and pH in the summer (Table 1). At the wetland outlet in Sanikiluaq, average concentration reductions of 56% for CBOD₅, 20% for TSS, and 3.4 log for *E. coli* were observed during the spring freshet. During the summer, average concentration reductions of 96% for CBOD₅, 93% for TSS, and 3.9 log for *E. coli* were observed

408 in the Sanikiluaq wetland. It can be noted that the effluent water quality generally improved as
409 the treatment season progressed. These findings tend to corroborate with observations of
410 seasonality in treatment performance observed in other tundra WTAs by Yates et al. (2012) and
411 Hayward et al. (2014). The average percent reductions from inlet to outlet during the spring in
412 Naujaat were 80% for CBOD₅, 48% for TSS, 0.04 log for *E. coli*; compared to an average
413 increase of 523% for CBOD₅, 21% reduction for TSS, 5 log reduction for *E. coli*, and a 60%
414 increase in NH₃-N during the summer. The elevated concentrations of organics and solids were
415 likely due to algae accumulation that was noted near the wetland outlet at the end of the
416 treatment season. Comparison between the two sites indicates that Sanikiluaq generally had
417 lower effluent concentrations for contaminants in comparison to Naujaat. In summary,
418 concentrations of most wastewater constituents decreased throughout both wetlands, except at
419 times in Naujaat. Effluent water quality tended to improve over the course of the treatment
420 season. Water samples collected at the outlet of both wetlands possessed elevated levels of
421 organic material, nutrients, and fecal indicator bacteria as compared to reference wetlands (Table
422 1).

Table 1. Minimum, maximum, and mean concentrations of water quality parameters for raw wastewater, wetland influent and effluent, and from reference wetland sites obtained during the spring freshet (Spring), and end of the summer treatment season (Summer). Mean concentrations are displayed in parentheses and when no range is reported the samples had the same value.

Site	Sample location	Sample size (n)	CBOD ₅ (mg/L)	TSS (mg/L)	<i>E. coli</i> (MPN/100 mL)	TN (mg/L)	NH ₃ -N ^a (mg/L)	TP (mg/L)	Temp. (°C)	DO (mg/L)	pH
Sanikiluaq	Water license requirement ^c		120	180	1x10 ⁶						6 – 9
	Raw	6	189 – 387 (306)	160 – 280 (219)	>6x10 ⁴ – >1x10 ⁶	79 – 123 (107)	<0.2 – 4.2 (0.9)	8 – 13 (11)	16 – 25 (20)	0.7 – 8.5 (2.8)	7.3 – 8.1 (7.7)
	Influent (Spring)	2	5 – 13	2 – 13	3.1x10 ⁴ – 6x10 ⁴	6 – 13	<0.2	1.4 – 1.7	1.2	0.3 – 0.7	7.4
	Effluent (Spring)	2	<4	4 – 8	10 – 27	0.6 – 1.2	<0.2 – 0.9	0.2	0.3 – 2.8	11.8 – 12.1	7.6
	Influent ^b (Summer)	2	23 – 250	72 – 93	2.3x10 ⁴	20 – 26	0.7 – 3.0	3.8 – 4.2	11 – 12	15 – 22	8.5 – 9.1
	Effluent (Summer)	2	<6	1 – 11	<3	0.6	<0.2	0.3 – 0.7	7	10 – 11	7.9 – 8.1
	Reference	6	<4 – <6 (9)	1 – 32 (9)	<3 – <10 (0.4)	<0.4 – 0.6 (0.4)	<0.2	<0.05	3 – 11 (7)	9.6 – 13.6 (11.4)	7.7 – 8.2 (8.0)
Naujaat	Water license requirement ^d		80	70	1x10 ⁶						6 – 9
	Raw	6	411 – 510 (462)	217 – 434 (324)	4.1x10 ⁶ – 1.7x10 ⁷ (1.2x10 ⁷)	84 – 149 (131)	0.9 – 3.5 (1.9)	10 – 19 (16)	19 – 25 (22)	4.5 – 6.7 (5.6)	7.6 – 8.1 (7.8)
	Influent (Spring)	2	85 – 125	44 – 57	9.5x10 ⁵ – 2.4x10 ⁶	33 – 40	0.42	4.1 – 4.7	5	5.8 – 6.1	7.6 – 7.8
	Effluent (Spring)	2	17 – 24	9 – 44	1x10 ⁵ – 1.7x10 ⁶	16 – 18	<0.2	2.1 – 4.1	4 – 6	1.6 – 9.7	7.4 – 7.6
	Influent (Summer)	2	7 – 17	48 – 64	4.6x10 ⁵ – 1.1x10 ⁶	17 – 40	0.4 – 1.2	2.8 – 2.9	7 – 12	9 – 12	8.0 – 8.3
	Effluent (Summer)	2	6 – 139	40 – 48	3 – 9	6.4 – 7.1	0.5 – 1.9	1.4 – 1.5	7.1 – 8.6	20 – 29	9.6 – 9.7
	Reference	5	<2 – <6	4 – 7 (6)	<1 – 21 (0.4)	<0.4 – 0.7 (0.4)	<0.2	<0.05	1 – 8 (4)	10 – 14 (13)	7.2 – 7.9 (7.5)

^aTN and NH₃-N were not determined for one of the two sample events in Naujaat during the spring period.

427   ^bThe summer influent sample was collected directly from the lagoon due to no surface flow conditions at the inlet in Sanikiluaq.

428   ^c Source: Nunavut Water Board (2015a). Specified in measurement units of BOD₅ (mg/L) and fecal coliforms in CFU/mL.

429   ^d Source: Nunavut Water Board (2015b). Specified in measurement units of BOD₅ (mg/L) and fecal coliforms in CFU/mL.

3.3. Distributions of gene target concentrations

The distribution of gene target absolute abundances and 16S rRNA absolute abundance in the raw wastewater and wetlands downstream of the wastewater disposal sites and reference wetlands are summarized in Figure 5. At both sites ARG concentrations were significantly higher in the spring in comparison to summer ($P < 0.05$) (except at the outlet in Sanikiluaq), and this seasonal variation is linked to the hydrology of the wetlands.

This indicates that the spring poses the greatest risk with respect to presence of elevated ARG concentrations in the environment, which corresponds with trends observed from measurements of standard wastewater quality indicators in other WTAs by Hayward et al. (2014) and Yates et al. (2012). The raw wastewater quality between the two sites ($n = 18$ gene targets) for both sampling seasons were comparable with no significant differences in the distribution of gene target concentrations (paired t-test, $t = 0.74$, $df = 8$, $p = 0.48$). There was significant difference ($P < 0.05$) in the distribution of gene target concentrations between the effluent and reference wetland sample locations during the treatment season in Sanikiluaq (paired t-test, $t = 2.6$, $df = 17$, $p = 0.02$). Likewise, in Naujaat there was a significance difference in the distribution of gene target concentrations between effluent and reference wetland sample locations during the treatment season (paired t-test, $t = 6.6$, $df = 17$, $p < 0.001$). Therefore, the distribution of gene targets at both wetlands did not return to baseline concentrations by the wetland outlets.

Figure 5. Distribution of absolute gene target abundances and 16S rRNA gene target absolute abundances at the sample locations in a) Sanikiluaq and, b) Naujaat. The stars on top x-axis are indicative of significant difference of distributions with paired student t-tests at $P < 0.05$. The middle lines represent the mean values, the bottom and top of the boxes represent the 25th and 75th percentiles, and the whiskers represent the 10th and 90th percentile of the distribution of gene targets. The raw data distributions were combined over the entire treatment season per site.

3.4. Absolute abundance in raw wastewater

Absolute abundance of each individual ARG target in the raw wastewater is shown in Figure 6. The gene targets with the highest prevalence in the wetlands were the class I integrase gene *int1*, *sul1*, *sul2*, and *qnrS* in Naujaat. These findings agree with previous findings from Narciso-da-Rocha et al. (2014) and Gaze et al. (2011) who observed that *int1* is particularly prevalent in wastewater. In addition, the sulfonamides (to which *sul* type genes confer resistance) group of antibiotics is one of the most commonly found in wastewater (Davies & Davies, 2010). More recently, the mostly plasmid-borne *qnrS* have been documented to be prevalent in wastewater sources, where it may be a concern for horizontal gene transfer events (Rodriguez-Moraz et al.,

2015). Although not shown in Figure 6, the gene *mecA* was present in the raw wastewater just slightly above the LOQ at $1.9 \log \text{ gene copies/mL} \pm 0.3 \text{ S.D.}$ and $2.0 \log \text{ gene copies/mL} \pm 0.2 \text{ S.D.}$ for Sanikiluaq and Naujaat, respectively. While, *vanA* was only observed above the LOQ once in one of the raw pump truck samples in Sanikiluaq.

Figure 7 shows the relative abundance of the distribution of ARGs in the raw wastewater were not significantly different between the two sites (paired t-test, $t = 0.96$, $df = 8$, $p = 0.36$). This could be attributed to similar strength and quality raw wastewater, and similar rates and types of clinical use of the antibiotics within the two hamlets. An exception to this trend can be observed with the *qnrS* gene target in Sanikiluaq, which was not present above LOQ in the raw wastewater.

3.5. Absolute abundance in wetlands

Absolute abundance for each individual ARG target at the sample locations within the wetlands is illustrated in Figure 6. The ARGs *ermB*, *tetO*, *bla_{TEM}*, and *bla_{CTX-M}* were all below the LOQ (but above the LOD only in the spring excluding the reference site) in the water samples collected from the mid-point, outlet and reference wetlands in Sanikiluaq. In contrast, *ermB*, *tetO*, *bla_{TEM}*, and *bla_{CTX-M}* were all present within the wetland during the spring period in Naujaat. At times there was limited log reduction in some ARGs as wastewater progressed through the wetland in Naujaat. The persistence of ARGs in Naujaat can be explained by the short HRT in the wetland in the spring, and lower dilution at this site compared to Sanikiluaq. At both sites, the spring conditions in all instances produced the highest gene absolute abundances within the wetlands.

The ARG *mecA* and *vanA* was generally at or below the LOQ levels in soil and water at both sites, therefore the individual plots for this ARG were not presented. However, it should be noted

mecA was detected in low concentrations of 2.9 log gene copies per gram in the soil at the mid-point, and concentrations of 2.1 log gene copies per mL in the effluent and reference wetland water in Naujaat. *MecA* and *vanA* are often present on mobile genetic elements in chromosomal DNA instead of plasmids (Biavasco et al., 2007; Colomer-Lluch et al., 2011) and therefore the low prevalence in the study wetlands may be due to low persistence of the bacteria carrying these genes.

Comparison of the findings between Sanikiluaq and Naujaat with the findings from Chaves-Barquero et al. (2016) on the Cambridge Bay lagoon and WTA provides broader contextual analysis of ARGs in wetland settings in Nunavut as shown in Table 2. In terms of the *sul* and *tet* markers, the Cambridge Bay site had similar values to Naujaat in this study during the spring. In terms of 16S rRNA markers, Cambridge Bay and Sanikiluaq were similar.

Table 2. Comparison of ARG absolute abundances in the wetland effluent from Cambridge Bay from Chaves-Barquero et al. (2016) and Sanikiluaq and Naujaat, Nunavut.

Gene target (log gene copies/mL)	Site				
	Cambridge Bay ^a	Sanikiluaq		Naujaat	
		Spring	Summer	Spring	Summer
Tetracycline resistance	3.8	0.6	-0.008	4.2	2.7
Sulfonamide resistance	5.6	2.9	-0.1	5.3	3.6
16S rRNA	7.3	7.1	6.7	9.7	9

^aResults from Barquero et al. (2016) study.

Figure 7 shows a decline in the relative abundance of ARGs which was generally observed from the inlet to the outlet of the wetland in Sanikiluaq. In Naujaat, the relative abundances decreased to a lesser extent within the wetland. Furthermore, during the summer there were increases of relative abundance in Naujaat (0.2 and 1.3 log gene copies per 16S rRNA genes for *int1*, and

qnrS, respectively), which indicated potential enrichment of these genes in the wetland during this period.

3.6. Absolute abundance in soil

The gene targets were also widely detected in the wetland soil samples (Figure 6). It should be noted that the soil samples were collected during the summer period. Although the water and soil concentrations are not directly comparable (CFU/mL vs CFU/g), the fact that the soil concentrations were multiple orders of magnitude greater for some ARGs (e.g., *sul1*, *sul2*, *tetO*, *qnrS*) may suggest that the soil acts as a sink, or that the soil provides a favourable biofilm environment conducive to ARG carrying bacteria proliferation. Soil reservoirs for antibiotic resistance and ancient antibiotic resistance elements residing in soil environments is a field of current study and remains a field requiring further study to protect human health (Allen et al., 2010). There were numerous instances whereby the gene absolute abundances were below the LOD and LOQ in the soil samples (Figure 6). At both sites, none of the soil samples contained levels of *bla_{CTX-M}* above the LOQ despite it being quantified in the Naujaat water samples from the wetland. This may weakly infer that this ARG, commonly found among members of Enterobacteriaceae (Narciso-da-Rocha et al., 2014), may not be associated with the bacterial communities in the soil biofilms.

Figure 7 shows the relative abundance of the gene targets in soil which were generally much lower than observed with the water samples. This would be expected given the greater microbial biomass and genetic material in the soil samples compared to the water samples.

525   Figure 6. Absolute abundance of ARGs in the raw wastewater (raw), influent (In), mid-point (Mid) and effluent (Out) water and soil
526   samples from the wetlands during spring and summer sampling periods.

528 Figure 7. Relative abundance of ARGs in the raw wastewater, and soil and water, in the wetlands during spring and summer sampling
529 periods. Note: as the dropdown bars become increasingly negative in the graphs, the gene target in question represents less of the
530 overall proportion of genes in the bacterial population (i.e., is less enriched).

3.7. Natural resistome

The absolute abundance of the gene targets in water were observed to be below the LOQ within the reference wetlands, with the exception of *int1*, *sul1*, *sul2*, and *bla_{CTX-M}* in Naujaat; however the concentrations were close to the LOQ (Figure 6). These ARGs that were observed in the water of the reference wetland in Naujaat were also detected in much greater absolute abundances within both the raw wastewater and within the treatment wetland, which would suggest that there is selective pressure associated with the wastewater, which may contribute to the proliferation of naturally occurring ARGs. In three cases ARGs were detectable at low levels in the soil of the reference wetlands, including *sul1*, *qnrS*, and *tetO* in Sanikiluaq, which may also suggest these genes are naturally present in the soil, and potentially not originating from anthropogenic sources at the reference sites. The reference wetlands generally had the lowest relative abundance for all samples at both study sites (Figure 7).

Recent research has begun to assess the possibility of anthropogenic impacts on AMR in remote and seemingly un-impacted marine and terrestrial environments. Anthropogenic impacts on global ARG distribution was investigated in remote Arctic marine waters by Tan et al. (2017), where the human mitochondrial gene target *Hmt* was found in remote high arctic marine sediment and correlated with elevated relative abundance of ARGs. Zhang et al. (2018) studied ARGs in relatively un-impacted (glacial soil and permafrost) and anthropogenic environments (river sediment), where it was observed that there was greater abundance and diversity in the anthropogenic environment, than the relatively un-impacted sites. With the far-reaching global spread of AMR, it is important to develop an understanding of the ancient resistome as baseline for change monitoring and the reference sites in the tundra wetlands provide an example of a relatively un-impacted environment. In addition, sites with naturally occurring ARGs could

contribute to the proliferation of ARGs given influxes of nutrients and environmental changes over time from anthropogenic impacts.

3.8. Correlations between ARGs and water quality indicators (WQI)

The PCA results with the WQIs and gene targets is presented in Figure 8a and illustrates that the gene targets were positively correlated with each other. Some gene targets were excluded from the PCA due to low or inconsistent detection levels across the hamlets (*mecA*, *vanA*, *qnrS*, *ermB*, and *bla_{CTX-M}*). Total coliform, *E. coli*, VSS, and Zinc were the other water quality parameters that were the most strongly correlated with the gene targets. This positive correlation between ARGs and total coliforms and *E. coli* is expected because the bacteria often carry the ARGs within their cellular structure in a selective environment, therefore a high number of bacteria and accordingly ARGs would be anticipated in wastewater streams. The correlation of ARGs with organic matter may also be related to elevated nutrients for the bacteria to consume and hence proliferate. The positive correlation of ARGs with zinc may be attributed to co-selection of ARGs and zinc which has been observed by Pal et al. (2015). Many of the other wastewater contaminants including TN, Cu, TAN, TP, Al, Fe, TSS, CBOD₅, and Mn had weak positive correlations with the gene targets which indicated that their persistence in the wetlands followed similar trends. Overall, the results of the PCA indicated that fecal indicators, such as *E. coli*, and organic matter (in the form of VSS) are possible indicators for elevated ARG levels downstream of municipal wastewater sources in tundra wetlands. Figure 8b shows the PCA scores plot from which it can be qualitatively noted that there were seasonal differences in the gene targets and WQI results, with the spring and summer sample scores grouped separately. Differences in concentrations based on the distance from the wastewater source were also observed, with sample scores of similar qualities grouped together.

Figure 8. a) Loadings plot of the PCA results, use of red text is for contrast; and (b) scores plot of the gene targets and WQI PCA results. Ellipses denote scores groupings of spring samples (green), summer samples (red), raw truck samples (blue), and reference samples (orange). Abbreviations indicate influent (I), mid-point (M), effluent (E), reference (R), spring (Sp), and summer (S).

581

582   3.9. First-order rate constants of ARGs

583   The first-order rate constants determined for the gene targets in the wetlands are summarized in
 584   Table 3. It should be noted that the k_{20} 's for a few of the gene targets (including *mecA* and *vanA*)
 585   were not determined (denoted by n/a in Table 3), due to absolute abundances being below LOD
 586   or below the LOQ within the wetlands or dilution from external hydrologic contributions.
 587   Overall, the first-order rate constants determined for the gene targets fell within the 40th to 95th
 588   percentile (ranging from 52 m/y for *sul1* to 1,549 m/y for *int1*) for Sanikiluaq, and within the 50th
 589   to 95th percentile (ranging from 81 m/y for *sul1* to 1,954 m/y for 16S rRNA) for Naujaat,
 590   compared to fecal coliforms measured in a group of FWS constructed wetlands compiled by
 591   Kadlec and Wallace (2009). The k_{20} 's were variable and unique for each gene target. This is a
 592   preliminary attempt at the assessment of first-order rate constants for ARG and other gene targets
 593   in wetlands impacted by municipal wastewater and further study should be conducted, especially
 594   due to the wide variability in k_{20} 's, to assess whether these compare to other cold climate
 595   treatment wetlands. These first-order rate constants are important parameters to inform design of
 596   passive treatment wetlands for ARG specific removal, as they describe the rates at which the
 597   wetlands attenuate the ARGs assessed and allow for sizing requirement calculations to inform
 598   the design process.

599   Table 3. First-order rate constants (k_{20}) for the absolute abundances of the gene targets and *E.*
 600   *coli* in the wetlands.

Site	<i>int1</i>	<i>sul1</i>	<i>sul2</i>	<i>qnrS</i>	<i>ermB</i>	<i>tetO</i>	<i>bla</i> _{TE} _M	<i>bla</i> _{CT} _{X-M}	16S rRNA A	<i>E. coli</i>
Sanikiluaq	k_{20} (m/y)									
	1549	52	428	n/a ^b	n/a	n/a	n/a	n/a	n/a	1592

	Percentile ^a										
	(%)	95	40	90	n/a	n/a	n/a	n/a	n/a	n/a	95
Naujaat	k_{20} (m/y)	203	81	108	106	1117	254	157	473	1954	146
	Percentile	80	50	60	60	95	80	70	90	95	70
	(%)										

^aPercentiles were where the k_{20} 's from this study fell in comparison to a distribution of k_{20} values for fecal coliforms from n = 47 wetland years for 23 wetlands FWS constructed wetlands summarized by Kadlec and Wallace (2009). The k_{20} 's from Kadlec and Wallace are based on calculation using nominal HRT.

^bThe k_{20} 's were not determined for *ermB*, *tetO*, *bla_{TEM}* and *bla_{CTX-M}* in Sanikiluaq because the ARG concentrations within the wetland were below LOQ. The k_{20} for 16S rRNA could not be determined for Sanikiluaq due to dilution from external hydrologic contributions.

4. Conclusions

This study demonstrated that the measured suite of nine ARGs were elevated above reference conditions at the wetland outlet at both sites, except for *mecA* and *vanA*. It was hypothesized that the hydrology of the systems would play a large role in the concentrations and spatial distribution of the ARGs within the wetlands, and this was supported by the data. Notably, the relatively short HRTs (< 2 days) during the high flow periods of the spring freshet produced the period with the highest ARG absolute abundance concentrations. This spring period can be viewed as a worst case scenario for ARG exposure risk within tundra wetlands impacted by municipal wastewater originating from continuous discharge systems. Overall, Sanikiluaq had improved levels of ARGs in comparison to Naujaat which may also have been linked hydrology and latitude differences. Elevated levels of ARGs in soil samples in comparison to the water samples collected in the summer period illustrated that the soils could either retain ARGs from a period of higher concentrations in the water, or may provide an environment conducive to

proliferation of bacteria that may carry the ARGs. The preliminary first-order rate constants were widely variable within the wetlands ranging from 52 m/y to 1,954 m/y ($\bar{x} = 587$ m/y) depending on the specific gene target. This study has provided the first assessment of ARG concentrations in tundra wetlands over an entire treatment season, to our knowledge the first assessment of the kinetics of ARG removal in these unique wetland systems, and comparative characterization of ARGs in un-impacted tundra wetlands.

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