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1 **The release of wastewater contaminants in the Arctic: A case study from Cambridge Bay,**
2 **Nunavut, Canada.**

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21

22 **Abstract**

23 The treatment of municipal wastewater in the Arctic is challenging due to a variety of
24 financial, operational, climatic and technical issues. To better understand the efficacy of current
25 wastewater treatment in this region and the hazard posed to receiving waters, we assessed the
26 occurrence of contaminants (i.e., pharmaceuticals, antibiotic resistance genes and nutrients) as
27 they moved through a lagoon-based treatment system in Cambridge Bay in Nunavut, Canada.
28 Wastewater treatment in this community is performed by the use of a lagoon-tundra wetland
29 system that is discharged into the marine environment and is representative of current common
30 practices throughout the region. In 2014, samples were collected before and during lagoon
31 discharge from two locations in the main lagoon, one location downstream from the lagoon
32 effluent and three locations offshore. Grab samples were collected to measure nutrients (e.g. total
33 nitrogen and phosphorus) and the presence of antibiotic resistance gene-bearing microbes, and
34 Polar Organic Chemical Integrative Samplers (POCIS) were deployed to collect passively
35 organic contaminants in all locations. A total of six pharmaceuticals were detected from a screen
36 of twenty-eight analytes during the study: atenolol, carbamazepine, clarithromycin, metoprolol,
37 sulfamethoxazole and trimethoprim. The greatest concentrations of nutrients, antibiotic
38 resistance genes (ARGs) and pharmaceuticals were found in sampling locations within the
39 treatment lagoon. Offshore of the release point, we observed limited to no detection of
40 pharmaceuticals and ARGs and no change in total nitrogen and phosphorus from pre-release. We
41 conclude that the current concentrations of monitored pharmaceuticals do not pose a significant
42 hazard at this time to aquatic organisms in Cambridge Bay.

43

44 **Keywords:** Arctic, pharmaceuticals, wastewater lagoons, risk assessment, nutrients

45

46 **Capsule statement:** Baseline exposure data for nutrients, antibiotic resistance genes and
47 pharmaceuticals in surface and seawater at Cambridge Bay is introduced, alongside an
48 ecotoxicological hazard assessment.

49

50 **1. Introduction**

51 Organic contaminants in wastewater effluents, including pharmaceuticals, are released to
52 aquatic ecosystems and have been found to pose a hazard under certain conditions to receiving
53 waters; this poses a challenge for current wastewater treatment practices in many regions (Fent et
54 al., 2006). Some pharmaceuticals such as anti-inflammatory drugs, antidepressants and
55 antibiotics are not completely eliminated in the human body and can therefore enter the sewage
56 system as parent compounds and their biologically active metabolites (Vasskog et al., 2009).
57 Many current wastewater treatment systems are not specifically designed to eliminate organic
58 contaminants and, as a consequence, many of these pollutants are able to persist through
59 wastewater treatment processes (Gunnarsdottir et al., 2013). In addition, monitoring actions for
60 most micropollutants have not been well established in most wastewater treatment facilities
61 (Bolong et al., 2009). Another concern is the presence of organisms that carry antibiotic
62 resistance genes (ARGs), which can threaten public health (Rowan, 2011). Antibiotic resistance
63 genes have been detected in the environment as a result of the prevalent human and veterinary
64 use of antibacterial and antimicrobial products (Kummerer, 2009). Nutrient enrichment has also
65 been a potential hazard to the aquatic environment with increasing eutrophication in freshwater
66 and enclosed marine systems downstream of areas of urbanization (Smith, 2003). Algal blooms
67 can block light from getting into the aquatic environment. With enough overgrowth, they can

68 prevent oxygen from getting into the water, thereby, endangering plants and animals. While the
69 releases of effluents have been characterized for many countries and regions of the world (Luo et
70 al., 2014), little to no work has been performed to quantify organic micropollutants and their risk
71 in polar regions because of the difficulties associated with travel logistics and sample holding
72 limitations. Therefore, the collection of sample replicates and comprehensive datasets suitable
73 for statistical analysis is highly constrained in these regions. As a consequence, there is a lack of
74 understanding of environmental risks, system performance and treatment mechanisms associated
75 with the treatment systems in polar regions (Chouinard et al., 2014).

76 Some studies have been performed in Arctic environments for the screening of
77 pharmaceuticals and personal care products in wastewaters. For example, Weigel et al. (2004)
78 studied the prevalence of selected pharmaceuticals in different sewage samples from Tromsø in
79 Norway as well as in the seawater from Tromsø-sound, the recipient of wastewater. The selected
80 pharmaceuticals were, among others, ibuprofen, and its metabolites, and the insect repellent
81 N,N-diethyl-3-toluamide (DEET) as well as caffeine, which was included as a tracer for
82 domestic sewage. Emnet et al. (2015) studied the occurrence of personal care products in two
83 Antarctic research stations, detecting six analytes in treated wastewaters, including the UV filters
84 4-methyl-bezylidene camphor, 2-hydroxy-4-methoxybenzophenone and 2,4-
85 dihydroxybenzophenone, the plastic monomer 2,2-bis(4-hydroxyphenyl)-propane, the steroid
86 hormone estrone and the antimicrobial triclosan. These compounds were detected at
87 concentrations comparable to those reported for international coastal waters adjacent to
88 significantly greater human populations (Balmer et al., 2005).

89 In many regions of the Arctic, the release of sewage with minimum or no treatment can
90 have consequences for the receiving environment due to high vulnerability of the Arctic

91 ecosystem to environmental contaminants (Gunnarsdottir et al., 2013). Kallenborn et al. (2008)
92 reported that pharmaceutical residues are degraded slower in Arctic environments compared to
93 release scenarios in lower latitudes. In their study a set of nine different antidepressants and their
94 transformation products were analyzed in receiving seawater from two locations in Norway, one
95 of them in a northern region. Increased environmental stability of these compounds was detected
96 in the Arctic environment compared to the temperate location. The removal of pharmaceutical
97 residues by photodegradation is limited during the Arctic polar night and the intensity of sunlight
98 (even continuously during periods of midnight sun) at other times of the year are less intense
99 than that of more temperate regions. Both limited photodegradation during the winter and the
100 cold Arctic climate can slow down the degradation rate of pharmaceutical residues in the
101 environment (Schwarzenbach et al., 2003).

102 Arctic communities frequently experience several challenges in order to perform adequate
103 treatment of their wastewaters. Characteristics such as geographical remoteness, adverse weather
104 and lack of basic services are common in many communities and make wastewater treatment,
105 whenever possible, a difficult task (Yates et al., 2012). The scarcity of accredited laboratories for
106 compliance testing and the necessity for trained personnel to manage wastewater facilities are
107 challenges that need to be overcome by these communities on a daily basis. The subsistence
108 fishery is a significant industry in many Arctic coastal regions, for which pollutant contamination
109 of marine species exploited for human consumption is a major concern. Exposure to
110 micropollutants and their uptake in the food web can have hazardous effects on human health
111 and the environment through bioaccumulation and biomagnification of chemicals (Gunnarsdottir
112 et al., 2013).

113 The Canada Health Act ensures that the majority of health services are publicly funded for
114 all Canadians, with administration occurring at the provincial and territorial level. Despite this
115 universality of health care, some differences occur in the health status of aboriginal and non-
116 aboriginal Canadians. First Nations populations experience greater rates of mental illness,
117 suicide, diabetes, asthma, cardiovascular disease, tuberculosis, hepatitis, syphilis and HIV/AIDS
118 than non-aboriginal populations (Romain, 2013). This can influence the amount of
119 pharmaceuticals that are needed to treat these diseases in Northern Canadian communities.

120 To begin to address the need for knowledge about wastewater contaminants exposure in
121 the Arctic, it is needed to quantify the types and quantities of nutrients and micropollutants in
122 lagoon discharge effluents and receiving waters. Such an effort would allow a partial
123 understanding of the possible hazards associated with wastewater discharges into receiving
124 environments. In this study, we examined the efficacy of wastewater treatment under arctic
125 conditions, by assessing the occurrence of selected wastewater contaminants attenuation and
126 release from a wastewater treatment facility in Cambridge Bay, Nunavut, Canada. Our objectives
127 were: first, to obtain recent exposure data for the wastewater contaminants in Cambridge Bay,
128 regarding to the concentrations of nutrients (total nitrogen and phosphorus), ARGs and
129 pharmaceuticals; and second, to provide a baseline of the current state of wastewater treatment in
130 Cambridge Bay, in anticipation of the eventual instalment, expected by 2017, of the Canadian
131 High Arctic Research Station (CHARS), a scientific facility for Arctic research, as well for
132 expanding populations in the Arctic in general. Of particular interest was the exposure data at the
133 water intake point that CHARS will eventually use for research purposes. We were also
134 interested on assessing the facility for evidence of any leaky sewage infrastructures, specifically
135 at Finger Bay. We hypothesize that the wastewater contaminants in Cambridge Bay do not pose

136 a significant risk at this time to the marine environment, and that the lagoon-wetland system in
137 this community has the ability to perform partial attenuation on nutrients, pharmaceuticals and
138 antibiotic resistance genes.

139

140 **2. Materials and Methods**

141 *2.1. Study location*

142 Cambridge Bay is located in the territory of Nunavut in the Canadian Arctic. It has a
143 population of approximately 1,400. Mean monthly temperatures range from a maximum and
144 minimum, respectively, of -28 °C and -35 °C in January to 13 °C and 5 °C in July (Government
145 of Canada, 2014). In 2017 CHARS will become operational. This will likely have an impact in
146 the community in terms of increases in population and use of water resources, including
147 wastewater disposal and treatment. This study provides a baseline for current wastewater impacts
148 prior to CHARS' opening.

149 The wastewater system monitored at Cambridge Bay is comprised of a wastewater lagoon,
150 formerly a series of natural lakes, that performs primary treatment and is discharged once a year,
151 during the summer, into a small hydrologically-isolated natural tundra wetland. Wastewater then
152 is released through an open channel into the marine environment. Municipal sewage from
153 household sewage tanks is regularly transported to the lagoon by sewage trucks that perform
154 dumping runs year-round.

155 *2.2. Sample collection*

156 The selection of sampling sites was done in consultation with local municipal authorities
157 with an overarching aim of characterizing the composition of wastewaters and receiving waters
158 for the target analytes. We were also interested in assessing risk to CHARS intake by wastewater

159 and the possibility of sewage leakiness into Finger Bay. Water was sampled from six selected
160 locations around the study site (Figure 1). These were: approximately 20 meters away from a
161 new wastewater drop off point (Lagoon Input 1); approximately 20 meters away from an older
162 drop off point (Lagoon Input 2); at the outflow of the natural tundra wetland (Wetland);
163 approximately 100 meters offshore of the primary discharge point (approximately 100 meters
164 from the Wetland site) to the bay (Outfall); at the seawater intake point for CHARS studies
165 (CHARS); and at a previously used run-off discharge point culvert, currently closed, located
166 around 300 meters west from the main discharge point (Finger Bay). No significant rain events
167 were registered during the sampling. Pharmaceuticals were passively sampled using triplicate
168 POCIS (Environmental Sampling Technologies, St Joseph, MO) in the “pharmaceutical”
169 configuration as described by MacLeod and Wong (2010). Sampling was performed both before
170 and during release of wastewater from the lagoon. Pre-release POCIS sampling was performed
171 from July 25 to August 8, 2014 for inland locations, and from July 26 to August 9 for offshore
172 locations. Wastewater release occurred from August 28 to September 5 with POCIS sampling
173 performed from August 29 to September 8 at all locations. Grab-sampling for nutrients
174 (composite sample) and antibiotic resistance genes (ARGs; triplicate sample) was conducted on
175 July 25 in the pre-release stage, and on September 3 during release. Field blanks immersed in
176 nanopure water (18 M Ω -cm, Millipore, Billerica, MA) in the appropriate containers were opened
177 during sampling to determine the extent of contamination. Samples were kept on ice within 24
178 hours after the sampling for transport to the local laboratory, and shipped on ice back to
179 Winnipeg for processing. Samples for nutrients were collected in 50 mL falcon tubes. Personnel
180 wore gloves disinfected with 70% isopropanol while handling ARGs samples which were
181 collected in autoclaved 500 mL polyethylene bottles pre-release (July 25) and during release

182 (September 3) from all sampling locations. Bottles were rinsed three times with sample water
183 before being filled to the top with no headspace. Field blanks filled with nanopure water (18
184 M Ω -cm, Millipore, Billerica, MA) in the appropriate containers were opened during sampling to
185 determine the extent of contamination. Samples were kept on ice within 24 hours after the
186 sampling for transport to the laboratory, where ARGs samples were filtered in a sterile
187 environment. These filters were kept at -20°C until shipment to the University of Strathclyde for
188 antibiotic resistance genes analysis.

189

190 *2.3. Determination of pharmaceuticals and nutrients*

191 We followed the methods of Carlson et al., (2013), for the analysis of pharmaceuticals.
192 Ultra high-performance liquid chromatography-tandem mass spectrometry (UHPLC/MS/MS)
193 with isotope dilution was used to quantify chemicals of interest in water samples. These
194 compounds included a suite of twenty-eight commonly-used micropollutants frequently found in
195 wastewaters (Fatta-Kassinos et al., 2011), including β -blockers (e.g. metoprolol); antidepressants
196 (e.g. fluoxetine); anticonvulsant drugs (e.g. carbamazepine) and macrolide (e.g. clarithromycin)
197 and sulfonamide (e.g. sulfamethazine) antibiotics. Limits of quantification (LOQ) are found in
198 Table S1, and other quality assurance/quality control parameters details in Carlson et al. (2013).
199 Time-weighted average (TWA) concentrations were calculated by dividing the determined mass
200 of chemical (ng) in the sampler by the sampling rate (L/d) times the deployment time (d) for all
201 detected pharmaceuticals, making use of sampling rates (Table S2) found in the literature
202 (MacLeod et al., 2007; Bartelt-Hunt et al., 2011). Concentrations of nutrients (e.g. total nitrogen
203 and total phosphorus) were determined before and during discharge following standard methods
204 (APHA, 2005).

205

206 *2.4. Hazard assessment for detected pharmaceuticals*

207 A hazard assessment was performed for detected compounds by calculating hazard
208 quotients (HQs) using standard tests and endpoints from aquatic toxicological studies,
209 particularly for primary producers, invertebrates and fish. Briefly, estimates for effective
210 concentrations (EC50) or lethal concentrations (LC50) were obtained through a literature search.
211 For added conservatism, we employed an uncertainty factor of 1000 to the lowest EC50 or LC50
212 (Waiser et al., 2012). The maximum measured environmental concentration was then divided by
213 the lowest reported effect concentration (typically freshwater, as marine organism tests were
214 lacking) to obtain the hazard quotient. Hazard quotients greater than 1 were considered to be of
215 concern while those compounds with HQ values less than 1 were considered less likely to pose a
216 concern.

217

218 *2.5. Determination of antibiotic resistance genes*

219 Antibiotic resistance genes were quantified from cells harvested on filters following cell
220 disruption (FastPrep, MP Biomedicals; 2 cycles at 30 s each at 6.0 setting) and DNA purification
221 (MoBio PowerClean Soil DNA kit; Cambio, Cambridge, UK), similar to methods previously
222 used (Anderson et al., 2015; Cardinal et al., 2014). A multiplex assay was used to target an array
223 of tetracycline resistant genes (Ng et al., 2001), sulfonamide resistant genes (Pei et al., 2006) and
224 16S-rRNA was quantified as a measure of ‘total bacteria’. Quantitative PCR was conducted
225 using a BioRad iQ cycler (BioRad, Hercules, CA) using ssoFast EvaGreen reagents (BioRad)
226 and 500 nM primer concentrations. All samples were diluted 1:100 with molecular grade water,
227 as reactions were predetermined to be most efficient at those sample concentrations; standards

228 and post-analytical melting curves were generated (Smith et al., 2004) to verify PCR reactions
229 quality and quantify results.

230

231 *2.6. Statistical analysis*

232 Changes in concentrations of pharmaceuticals, as well as abundance of ARGs, from before
233 and during release were assessed using a Student's paired *t*-test. Concentration data are presented
234 as mean \pm standard deviation (St. Dev) unless otherwise indicated. Differences were considered
235 significant at $p < 0.05$.

236

237

238 **Results and Discussion**

239 *3.1. Nutrients*

240 Nutrient samples had to be combined in order to measure concentrations at many sites,
241 particularly those offshore given very low levels there (Table 1). Thus, only a single
242 measurement was made per site per sampling time, so our discussion of changes in
243 concentrations is qualitative in nature.

244 Total nitrogen showed values over 10 mg/L for the lagoon sites both before and during
245 release measurements. Concentrations at Lagoon Input 1 and Lagoon Input 2 showed similar
246 levels at both times, with the Wetland site having an increase in concentration after the
247 wastewater discharge started. There was no apparent reduction in the concentration of nitrogen in
248 the lagoon or the wetland in the two time periods. Locations offshore (e.g. Outfall, CHARS)
249 showed much lower concentrations. Total phosphorus levels were approximately 2 mg/L for
250 both lagoon sites prior to wastewater discharge. After the discharge commenced, phosphorus

251 levels in the wetland were elevated to approximately 2 mg/L as well, with no apparent reduction
252 from additional wetland treatment. Phosphorus levels appear to be greater and nitrogen values
253 appear to be lesser than the maximum values recommended for Canadian provinces such as
254 Manitoba, in which limits of 1 mg/L total phosphorus and 15 mg/L total nitrogen exist for
255 wastewater effluents discharged to a water body (Manitoba Water Stewardship, 2011). However,
256 policies for communities in the far north have not yet been defined and a joint governmental
257 commission has been assigned to define them by 2019 (CCME, 2014). Considerable dilution was
258 observed for all locations offshore (e.g. Outfall, CHARS), which was consistent with the
259 nitrogen measurements. Finger Bay showed reduced levels for both total nitrogen and
260 phosphorus, which suggests that there is little possibility of runoff from the main lagoon to this
261 location contrary to prior speculations that this was a route of contamination from the lagoon to
262 the bay. The levels of phosphorus we measured pre-release are comparable to that in the water
263 column at the center of Cambridge Bay and at Dease Strait, a waterway immediately west of
264 Cambridge Bay (0.01-0.04 mg/L, C. J. Mundy, unpublished data). Concentrations of phosphorus
265 at the Outfall site are roughly twice those levels, suggesting localized effects of phosphorus that
266 are not evident at points farther away in the bay (Table 1). While nutrient levels during release
267 are likely locally elevated relative to concentrations in the greater Canadian Arctic (Tremblay et
268 al., 2015), more work is warranted to examine to what extent these added nutrients may
269 influence the local ecosystem of Cambridge Bay and Dease Strait.

270 No apparent nutrient removal was observed during discharge as a result of lagoon-wetland
271 treatment. As noted, statistical analysis of nutrient concentrations was not possible. Nor can we
272 rule out the possibility that nutrient concentrations may have been affected by heterogeneous
273 distributions within different locations of the lagoon. That having been said, the data obtained in

274 this study differ from the results obtained in a previous work by Yates et al. (2012), in which
275 three larger lagoon-wetland systems in Nunavut (Arviat, Whale Cove and Coral Harbour) were
276 studied, observing reductions up to 84-99% for NH₃-N and 80-99% for total phosphorus. It is
277 known that the community of Arviat make use of berms and channels to direct wastewater flow
278 away from the ocean and to keep a longer residence time in the wetland (Wooton et al., 2008),
279 whereas in the Cambridge Bay wetland the residence time of wastewater is limited by the
280 landscape topography and the scarce available vegetation. It is yet unclear which mechanisms
281 play the most important role in wastewater treatment in the Arctic. Wetland size and vegetation
282 coverage as well as the potential for filtration and sedimentation of suspended solids and
283 adsorption of nutrients within the soil and water column can play a significant role.

284

285 *3.2. Pharmaceuticals*

286 Of the screened twenty-eight organic micropollutants, only six pharmaceuticals were
287 detected above their LOQ at any of the locations. These were atenolol, clarithromycin,
288 metoprolol, sulfamethoxazole, trimethoprim and carbamazepine, detected at least once in ng/L
289 levels (Figure 2). For the detected pharmaceuticals, the greatest concentrations were measured at
290 the Lagoon Input 1 and 2 sites, although some differences in concentration could be seen
291 between both of the dumping sites. Most locations offshore experienced considerable dilution
292 with seawater, which was reflected in significantly lower concentrations for all of the passively
293 sampled contaminants at Outfall, CHARs and Finger Bay. Although POCIS deployment times
294 were different before and during discharge (14 days versus 9 days), steady state conditions for
295 POCIS are typically reached within six days (Vermeirssen et al., 2012)

296 The greatest concentration of atenolol was 97 ng/L (Lagoon Input 1). Detected levels were
297 significantly different ($p < 0.05$) between Lagoon Input 1 and Lagoon Input 2 sites both before
298 and during the discharge. There was a significant reduction of 45% observed between Lagoon
299 Input 2 and Wetland ($p < 0.05$) during the wastewater release, and heavy dilution at locations
300 offshore, with most sites observed to be at non-detectable levels. These results suggest processes
301 within the wetland (e.g. sorption to plants, microbial degradation) may reduce concentrations of
302 atenolol under Arctic conditions. A more efficient removal of atenolol has been observed in
303 more southern locations in Canada, with removal rates up to 98% for example within a sewage
304 lagoon in Dunnottar, Manitoba under temperate conditions (Anderson et al., 2015).

305 Concentrations of carbamazepine were generally below 100 ng/L in both lagoon sites, with
306 greater concentrations reported in the Wetland site both before and during discharge. No
307 apparent removal was observed as a result of wastewater passage through the treatment wetland
308 ($p < 0.05$). Offshore locations showed levels below LOQ. Persistence of carbamazepine during the
309 Arctic winter was observed, with a concentration of 116 ng/L in the hydrologically isolated
310 wetland prior to discharge. While no measurements of pharmaceuticals occurred during the
311 winter, we note that this shallow wetland system and the offshore locations are predominantly or
312 completely frozen over the winter. This would presumably result in no removal of analytes by
313 either microbial activity or photodegradation (i.e., light penetration would be prevented almost
314 completely by ice cover) until summer melt.

315 The greatest concentration of sulfamethoxazole was 274 ng/L; this was detected at the
316 Lagoon Input 2 site during wastewater discharge. Concentrations between lagoon sites were
317 significantly different both before and during discharge ($p < 0.05$), with some attenuation
318 observed after wetland treatment, reaching 151 ng/L (45% removal, $p < 0.05$). Levels offshore

319 were non-detectable both before and during discharge. Unlike this study, Conkle et al. (2008)
320 noted over 90% removal of sulfonamides on a temperate wastewater facility, however, the
321 differences may have been as a result of significantly greater temperatures and a 27-day retention
322 period compared to a drastically colder weather and shorter retention time at Cambridge Bay
323 facility of 1-2 days

324 Trimethoprim was detected in concentrations under 30 ng/L at the lagoon and wetland
325 sites. During wastewater release, the wetland concentration was 9.8 ng/L after significant
326 attenuation ($p < 0.05$) occurred between the lagoon and the wetland. Finally, clarithromycin and
327 metoprolol were detected at both of the lagoon sites and also in the wetland at levels below LOQ.
328 At the offshore sites, both compounds were non-detectable, which is consistent with what was
329 observed for all contaminants studied.

330 The presence or absence of specific pharmaceuticals depends partially on the residence
331 time within sewage holding tanks, prior to entry into sewage lagoons. While photodegradation is
332 unable to occur in septic tanks, other degradative processes like anaerobic microbial-mediated
333 biotransformation could occur. Consequently, the most labile compounds were likely partially
334 degraded to an unknown extent. Sorption of pharmaceuticals to septic tank particulates may also
335 occur. Photodegradation and biotransformation are typically the most important processes for the
336 attenuation of organic micropollutants in effluent-receiving waters. Consequently, optimization
337 of conditions for these processes (e.g. by using extended periods of treatment in sewage lagoons)
338 can effectively minimize or prevent environmental exposure to biologically active levels of these
339 contaminants (Ying et al., 2009).

340 To the best of our knowledge, there are no reported data for pharmaceuticals from
341 wastewater systems from Northern Canada. Nevertheless, a larger amount of information (Table

342 3) is available for treated lagoon wastewaters using passive sampling of more southern regions in
343 Canada, including various works done in the province of Manitoba (Anderson et al., 2013;
344 Anderson et al., 2015; Carlson et al., 2013) and Alberta (MacLeod and Wong, 2010). At
345 Cambridge Bay, all detectable compounds had greatest concentrations at either the lagoon or the
346 wetland sites and were mostly non-detectable at locations offshore. Atenolol, carbamazepine,
347 sulfamethoxazole and trimethoprim were detected in the Cambridge Bay facility at lower levels
348 compared to the data obtained at Dunnottar, Manitoba (Anderson et al., 2015). On the other
349 hand, atenolol, sulfamethoxazole and trimethoprim were detected in greater levels than in the
350 Grand Marais wastewater treatment facility. Levels of drugs were similar in wastewaters of
351 Cambridge Bay and Lac la Biche, Alberta (MacLeod and Wong, 2010).

352 There are several factors that likely account for the differences in pharmaceutical levels
353 among these locations. One factor is population, with greater populations implying greater
354 loadings and impact on wastewater release. The populations served by the treatment facilities of
355 the southern Canadian sites (Table 3) are all on the order of several thousand, with some
356 seasonal variability. For example, the Dunnottar population, a popular regional summer resort
357 (Anderson et al., 2015) is several times greater than that of Cambridge Bay during the summer.
358 However, similar per capita use of drugs may result in similar concentrations in wastewaters
359 (MacLeod and Wong, 2010), which appears to be the case based on our comparisons (Table 3).
360 Another factor is temperature, given the fact that colder temperatures in Nunavut can cause
361 treatment mechanisms such as sorption to be slower and less efficient when compared to
362 temperate locations, as it has been previously observed in Norway (Kallenborn et al., 2008).
363 Both factors are likely in play, confounding prediction of pharmaceutical levels in wastewaters.
364

365 3.3. Risk assessment for detected pharmaceuticals

366 Hazard quotients (HQs) were calculated for each organic contaminant based upon toxicity
367 data reported in the literature for primary producers, invertebrates and fish (Table S3). Most
368 compounds had an HQ less than 1, ranging from values between 10^{-6} and 10^{-1} . Only
369 clarithromycin presented an HQ greater than 1 for the algae *Pseudokirchneriella subcapita*,
370 which indicates that there is a potential for growth inhibition of algal species at concentrations
371 such as those detected in lagoon and in the wetland. While the HQ for clarithromycin was greater
372 than 1, the concentration level used for calculation may not be necessarily representative of what
373 could be found in the entire lagoon, the use of 1000-fold uncertainty factor adds a high degree of
374 conservatism. As well, in eutrophic environments, such as these lagoons, excess nutrients can
375 mitigate the effects of compounds that exhibit herbicidal activity (Baxter et al., 2013). For the
376 rest of the detected pharmaceuticals, we can conclude that there is likely no significant hazard to
377 aquatic life due to the low concentrations at which they were detected. We did lack Arctic and
378 marinespecific tests that would reduce the uncertainty and did not assess for the effect of
379 mixtures of chemical stressors. We do recommend the development of standard toxicity tests
380 with Arctic marine organisms to help address this uncertainty

381

382 3.4. Abundances and removal of ARGs

383 Total bacterial populations were determined by means of the abundances of 16S rRNA
384 genes. Their presence was greatest in the lagoon sites in both sampling periods: before the
385 wastewater discharge started ($10^{8.0}$ genes/mL in Lagoon Input 1 and $10^{7.8}$ copies/mL in Lagoon
386 Input 2), and during wastewater discharge ($10^{7.4}$ genes/mL in Lagoon Input 1 and $10^{7.5}$
387 copies/mL in Lagoon Input 2) (Table 2). Overall, the abundances of 16S rRNA genes were

388 similar (i.e., differences found were not greater than one order of magnitude) to levels reported at
389 more southern locations in Canada (Anderson et al., 2015). Comparing concentrations before and
390 during wastewater discharge, gene abundances did not change significantly (paired t-test, $t_5 = -$
391 1.46, $p = 0.203$) and their distribution pattern remained similar ($r = 0.965$, $p = 0.002$) along the
392 waste stream.

393 Clusters of tetracycline resistance and sulfonamide-resistance genes were analyzed and the
394 results were summed to facilitate assessment of resistance patterns. The greatest abundances of
395 tet^R (sum of tetracycline resistance genes) and sul^R (sum of sulfonamide resistance genes) were
396 found in the primary lagoon, at the two wastewater drop-off locations, being Lagoon Input 2 the
397 one with the greatest response, with some attenuation through the wetland (Table 2).

398 Specifically in terms of the tetracycline resistance genes, differences from lagoon levels to
399 offshore levels were around one order of magnitude during discharge, with locations after the
400 wetland having reductions most likely due to dilution. Outfall was the sampling spot with the
401 lower amount of tet^R genes before and after discharge. Distribution of concentrations before and
402 during discharge was similar ($r = 0.941$, $p = 0.005$), but became lower after discharge (paired t-
403 test, $t_5 = 3.66$, $p = 0.015$) quite possibly due to dilution with water from the environment (e.g.
404 existing surface water and/or groundwater seeps)

405 Sulfonamide resistance genes were more highly concentrated in the lagoon and wetland,
406 and before discharge declined rapidly (2-3 orders of magnitude) following the wetland. During
407 discharge, gene concentrations were variable at the two drop-off points in the lagoon, with
408 minimum to no attenuation from the wetland. Gene distribution patterns along the waste stream
409 were comparable (before-during discharge; $r = 0.887$, $p = 0.019$), but unlike 'total tet' there

410 were significant pairwise changes (paired t-test, $t = 0.506$, $p = 0.634$) as most concentrations
411 decline, except at the wetland and slightly in CHARS site.

412 To facilitate further analysis in prevalence of bacteria throughout the treatment process,
413 abundances of resistance genes were divided by the abundance of 16S rRNA genes to represent
414 relative gene abundances. Relative abundances of ARGs were low (e.g. less than 2% of the total
415 as observed in the wetland) at all locations during discharge (see Table 2), which suggests a low
416 potential for ARG-bearing bacteria to exist throughout the treatment system. Tetracycline
417 resistance genes remain elevated in wastewater systems if there is a source of resistance
418 microorganism and tetracycline usage (Peak et al., 2007), but can decline in sunlight-exposed
419 systems over a relatively short period of time (Engemann et al., 2008; Zhang et al., 2009). This
420 suggests that tetracycline may not have been extensively used in the Cambridge Bay population
421 around the times of sampling. Gene concentrations were equivalent to wastewater lagoons with
422 minimal tetracycline usage by source population e.g., (Peak et al., 2007), and could already
423 represent near background levels (Engemann et al., 2006; Zhang et al., 2009). Whereas,
424 detectable sulfonamide concentrations in this study may have been sufficient to maintain
425 selective pressure for antibiotic-resistant bacteria, or their presence of elevated levels represent
426 residual evidence of previously higher levels of sulfonamide usage, as gene fate tends to differ
427 from chemical fate (e.g. Engemann et al., 2006; Peak et al., 2007)

428 Wastewater systems have a variable ability to reduce antimicrobial resistance, given the
429 fact that generally resistant bacteria numbers decline in wastewater treatment as bacteria are
430 removed, but these patterns require further investigation, as they remain a function of bacterial
431 community and operating conditions (Christgen et al., 2015). Further, no studies to date have
432 examined the fate of antibiotic resistant bacteria in the wastewater stream at lower temperatures,

433 such as in the Arctic. Well-studied coliform bacteria, which tend to carry ARGs, persist longer in
434 colder temperatures (Solic and Krstulovic, 1992); however, gene persistence at lower
435 temperatures could be exacerbated by slowed transformation rates of pharmaceutical compounds
436 and prolonged selective pressures, reduced endonuclease activity, and lowered predation. Further
437 investigations are required to fully elucidate gene fate under psychrophilic conditions.

438

439 **Conclusions**

440 Our assessment of the Cambridge Bay wastewater treatment facility allowed us to detect
441 no apparent removal of nutrients as a result of lagoon-wetland treatment. Reduced nutrients
442 concentrations at locations offshore occurred as a result of dilution. Our data suggests that some
443 attenuation mechanisms for pharmaceuticals exist in the treatment system, especially in the
444 sewage lagoon and to some extent in the natural wetland. Distribution of the wastewater
445 contaminants within the lagoon sites was not homogeneous, due to the presence of two different
446 drop off points for sewage dumping and the topography of the lagoon. From all of the studied
447 pharmaceuticals, only carbamazepine showed some persistence during the Arctic winter.
448 Atenolol, sulfamethoxazole and trimethoprim had dissipated prior to the first sampling
449 campaign. Concentrations of detected pharmaceuticals and nutrients were minimal in the Finger
450 Bay location, which suggests that there was minimal runoff of wastewater to this point. Hazard
451 assessment for detected pharmaceuticals shows that current concentrations of monitored
452 pharmaceuticals do not pose a significant hazard at this time to aquatic organisms in Cambridge
453 Bay. Bacterial populations were detected in similar levels to more southern Canadian locations,
454 with some ARGs attenuation observed in the lagoon-wetland system and considerable dilution at
455 locations offshore. Finger Bay experienced non-detectable levels for all pharmaceuticals and

456 very low levels of ARGs, which suggests that this location was not likely experiencing any
457 sewage leaking at the time of this study. Overall, the CHARS scientific water supply location
458 showed non-detectable levels for all pharmaceuticals and very low levels of ARGs, prior to the
459 instalment of the facility at Cambridge Bay. This study constitutes one of first attempts ever
460 made to understand the occurrence of pharmaceuticals, ARGs and nutrients on wastewater
461 treatment facilities in the Canadian Arctic, as well as the removal performance of these systems
462 under polar conditions.

463

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475 **Table 1**

476 Levels of total phosphorus and total nitrogen in the Cambridge Bay wastewater facility before
 477 and during wastewater discharge (n=1). Wastewater was grab-sampled on July 25 and September
 478 3, 2014. The limit of detection (LOD) and limit of quantification (LOQ) values for total nitrogen
 479 were 8 µg/L and 27 µg/L, and those for total phosphorus were 0.58 µg/L and 1.85 µg/L,
 480 respectively.

Sampling site	Before discharge		During discharge	
	Total phosphorus (mg/L)	Total nitrogen (mg/L)	Total phosphorus (mg/L)	Total nitrogen (mg/L)
Lagoon Input 1	2.4	12.6	2.8	12.0
Lagoon Input 2	2.4	16.6	2.8	13.5
Wetland	0.7	0.4	2.5	13.3
Outfall	0.01	0.3	0.07	0.4
Finger Bay	0.01	0.3	0.03	0.3
CHARS	0.02	0.4	0.03	0.3

481

482

483

484 **Table 2**

485 Abundances of antibiotic resistance genes ($\log(\text{genes/mL})$) harvested from grab-samples taken at
 486 the Cambridge Bay wastewater treatment facility and receiving waters in 2014; standard
 487 deviation of sample replicates (n=3) are denoted in parentheses. Relative abundance of ARGs to
 488 16S rRNA, calculated during discharge (A = tetR/16S rRNA ratio, B = sulR/16S rRNA ratio) is
 489 also shown.

Sampling site	Before discharge			During discharge			A (%)	B (%)
	log total tet ^R	log total sul ^R	log 16S rRNA	log total tet ^R	log total sul ^R	log 16S rRNA		
Lagoon Input 1	4.2 (0.3)	4.7 (0.5)	8.0 (0.5)	3.8 (0.2)	4.4 (0.3)	7.4 (0.4)	0.02	0.1
Lagoon Input 2	4.3 (0.3)	6.0 (0.7)	7.8 (0.9)	4.1 (0.3)	5.6 (0.3)	7.5 (0.8)	0.03	1.3
Wetland	3.9 (0.2)	4.4 (0.3)	6.8 (0.4)	3.8 (0.2)	5.6 (0.3)	7.3 (0.6)	0.03	2.0
Outfall	2.9 (0.2)	2.8 (0.2)	5.4 (0.4)	2.7 (0.2)	1.8 (0.1)	6.1 (0.4)	0.04	0.01
Finger Bay	3.0 (0.1)	2.8 (0.2)	5.3 (0.2)	2.7 (0.2)	2.0 (0.2)	6.3 (0.4)	0.02	0.01
CHARS	3.7 (0.2)	3.0 (0.2)	5.2 (0.4)	3.0 (0.3)	3.3 (0.3)	6.3 (0.4)	0.05	0.1

490

491 **Table 3**
 492 Comparison of concentrations of target pharmaceutical compounds in treated wastewaters of
 493 different Canadian lagoon wastewater systems (NA: not analyzed, ND: non-detectable). Lac la
 494 Biche data from MacLeod and Wong (2010), Dunnottar data from Anderson et al. (2015), Grand
 495 Marais data from Anderson et al. (2013), Cambridge Bay, this study. Populations are shown
 496 underneath the name of each location for comparison.

Location	Atenolol (ng/L)	Carbamazepine (ng/L)	Sulfamethoxazole (ng/L)	Trimethoprim (ng/L)
Lac la Biche, AB (8,402)	ND - 100	50 - 300	NA	10 - 15
Dunnottar, MB (692)	ND - 856.5	20.1 - 426.1	ND - 1252.5	ND - 318.5
Grand Marais, MB (252)	ND	85-500	ND-21	ND
Cambridge Bay, NU (1,400)	ND - 97.4	1.2 - 306.7	ND - 274.2	ND - 25.7

497

498

499 **Figure captions**

500

501 **Figure 1.** Sampling site locations at the Cambridge Bay wastewater treatment facility and
502 receiving waters. Wastewater path from the lagoon to the bay is shown by means of a dotted line.

503

504 **Figure 2.** Mean levels of pharmaceuticals in Cambridge Bay wastewater facility, before and
505 during the wastewater discharge process (n=3). Error bars depict the standard deviation on each
506 case. Differences are significant for $p < 0.05$ and were assessed using paired t-tests. Wastewater
507 was collected using Polar Organic Chemical Integrative Samplers from July 25 to August 8,
508 from July 26 to August 9 and from August 29 to September 8, 2014. TWA: time-weighted-
509 average.

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