Environmental Science
Processes & Impacts

RSC Publishing

PAPER

Fecal pathogen pollution: sources and patterns in water and sediment samples from the upper Cook Inlet, Alaska ecosystem

Cite this: *Environ. Sci.: Processes Impacts*, 2013, **15**, 1041

Stephanie A. Norman,[a] Roderick C. Hobbs,[b] Stefan Wuertz,[cd] Ann Melli,[a] Laurel A. Beckett,[e] Nadira Chouicha,[a] Arti Kundu[c] and Woutrina A. Miller*[a]

Fecal pathogens are transported from a variety of sources in multi-use ecosystems such as upper Cook Inlet (CI), Alaska, which includes the state's urban center and is highly utilized by humans and animals. This study used a novel water quality testing approach to evaluate the presence and host sources of potential fecal pathogens in surface waters and sediments from aquatic ecosystems in upper CI. Matched water and sediment samples, along with effluent from a municipal wastewater treatment facility, were screened for *Salmonella* spp., *Vibrio* spp., *Cryptosporidium* spp., *Giardia* spp., and noroviruses. Additionally, *Bacteroidales* spp. for microbial source tracking, and the fecal indicator bacteria *Enterococcus* spp. as well as fecal coliforms were evaluated. Overall, *Giardia* and *Vibrio* were the most frequently detected potential pathogens, followed by *Cryptosporidium* and norovirus, while *Salmonella* was not detected. Sample month, matrix type, and recent precipitation were found to be significant environmental factors for protozoa or host-associated *Bacteroidales* marker detection, whereas location and water temperature were not. The relative contribution of host-associated markers to total fecal marker concentration was estimated using a Monte Carlo method, with the greatest relative contribution to the *Bacteroidales* marker concentration coming from human sources, while the remainder of the universal fecal host source signal was uncharacterized by available host-associated assays, consistent with wildlife fecal sources. These findings show how fecal indicator and pathogen monitoring, along with identifying contributing host sources, can provide evidence of coastal pathogen pollution and guidance as to whether to target human and/or animal sources for management.

Received 29th November 2012
Accepted 21st March 2013

DOI: 10.1039/c3em30930d

rsc.li/process-impacts

Environmental impact

The Cook Inlet watershed, the major urban center of Alaska, is the fastest growing region in the state. The presence of potential pathogens, introduced from terrestrial sources through stormwater and urban run-off into waterways entering the marine environment, poses potential threats to ocean, human, and animal health. Environmental monitoring of water and sediment was used to evaluate the presence of potential fecal pathogens in the environment. Fecal indicator bacteria and coliforms were evaluated as well as microbial source tracking, to determine relative host contribution to fecal marker concentration. The greatest relative contribution to the *Bacteroidales* marker concentration was from human sources. These monitoring tools can provide evidence of coastal pathogen pollution and guidance on whether to target human and/or animals sources for mitigation.

Introduction

The Cook Inlet (CI) watershed covers 47 000 square miles of southcentral Alaska that drains into Cook Inlet.[1] The CI region is the major urban center in Alaska, with over 435 000 people, or approximately 2/3 of the state's population, residing in the watershed, and is the fastest growing region in the state.[2] With CI communities and the endangered CI beluga (*Delphinapterus leucas*) population dependent on a healthy watershed for sustainability, concern has grown among stakeholders regarding the inlet's water quality as it pertains to human health and that of wildlife inhabiting this region. The presence of potential pathogens, introduced from terrestrial sources

[a]*Department of Pathology, Microbiology & Immunology, School of Veterinary Medicine, University of California, Davis, CA 95616, USA. E-mail: wamiller@ucdavis.edu; Fax: +1-530-752-3349; Tel: +1-530-219-1369*

[b]*National Marine Mammal Laboratory, Alaska Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Seattle, WA 98115, USA*

[c]*Singapore Centre on Environmental Life Sciences Engineering, School of Civil and Environmental Engineering, Nanyang Technological University, 60 Nanyang Drive, Singapore 637551*

[d]*Department of Civil and Environmental Engineering, University of California, One Shields Avenue, Davis, CA 95616, USA*

[e]*Division of Biostatistics, Department of Public Health Sciences, School of Medicine, University of California, Davis, CA 95616, USA*

through stormwater and urban run-off into waterways entering the marine environment, poses potential threats to ocean, human, and animal health.[3]

Based on their habitat and dietary preferences, endangered CI belugas may be exposed to potential pathogens from multiple point and non-point sources.[4] In the CI region, the National Marine Fisheries Service (NMFS), a federal agency tasked with overseeing management and recovery of CI belugas, designated critical marine and estuarine habitat considered essential for the survival of the CI belugas. This critical habitat encompasses most of the upper inlet, including freshwater and marine areas draining watersheds including our study sites. However, NMFS identified large gaps in the data on occurrence and trends of fecal pathogens in beluga habitat (National Marine Fisheries Service, unpubl. data). In light of the beluga population's decline and lack of recovery, there is interest in investigating the presence and distribution of terrestrial source fecal microbes within coastal areas designated as critical habitat for these whales.[4] Morbidity and mortality due to fecal pollution has not yet been definitively demonstrated in this stock; however, based on reports in other beluga populations, fecal pathogens such as *Vibrio* spp. may pose a threat to the health and viability to this endangered stock.[5] Additionally, fecal protozoa have been detected in marine waters and survive well in this environment, which may have implications for human waste discharged from municipal sources and boat wastewater, as well as for coastal wildlife sources.[6]

Relatively little is understood about the ecology and sources of fecal microbes entering nearshore aquatic environments within CI, though it is recognized that fecal by-products from humans, their associated animals, and wildlife may affect the quality of water and food resources in coastal ecosystems.[7] Terrestrial sources of fecal waste entering aquatic ecosystems include wild and domestic animal-derived fecal matter carried in storm runoff, runoff from snowmelt and surface waters, and effluent from sewage outfalls.[8,9] Water entering from the local primary wastewater treatment facilities may contain a variety of organic and inorganic pollutants including metals, nutrients, sediments, drugs, bacteria, protozoa, and viruses that may end up in the inlet.[10] Monitoring for the presence of fecal microbes along with identifying contributing human or animal host sources within CI and its feeder rivers may provide important insights into the extent of fecal microbial pollution and how management strategies may be prioritized, as well as identifying adverse coastal ocean health events and potential impacts on CI belugas.[11]

The goal of this study was to investigate the presence and distribution of potentially pathogenic bacteria and protozoa, as well as fecal indicator bacteria (FIB), in marine and freshwater samples from eight targeted sites within upper CI. It has been noted that concentrations of fecally derived microbes are at times greater in sediment than water, thus both water and sediment were sampled at study sites.[12] The study hypothesis was that season, sampling location, sample matrix, water salinity and temperature, and timing of precipitation (within or greater than 7 days prior to sampling) would be associated with increased odds of detecting fecal microbes in environmental samples. The data collected in this study provide a baseline microbial dataset and identify relative host-associated contributions for environmental samples that can be used to help detect emerging risks to human and animal health, as well as for prioritizing mitigation strategies.

Materials and methods

Study sites and sample collection

A cross-sectional sampling of eight sites in upper CI was performed in the early and late summer seasons, including the Anchorage Water and Wastewater Utility (AWWU) effluent originating from the primary treatment plant ("effluent"), intertidal water around the AWWU outfall site, and matching water column and sediment samples from select urban and rural rivers and creeks in communities surrounding Anchorage (Fig. 1 and Table 1). The following samples (number of water/number of sediment) were collected at each site: Peters Creek (2/2), Eagle River (2/2), Ship Creek (3/2), Goose Creek (2/2), Chester Creek (3/3), Fish Creek (3/3), intertidal zone (4/4), and effluent (4/—). A set of 23 samples (13 water and 10 sediment) was collected during the first week of June and 18 samples (10 water and 8 sediment) during the last week of August 2011, comprising fresh, estuarine, and marine water types (Table 1).

Target microbes included the potential fecal pathogens *Salmonella* spp., *Vibrio* spp., *Cryptosporidium* spp., *Giardia* spp., and norovirus. Anaerobic bacteria specific to human or other animal host sources were quantified, using *Bacteroidales* qPCR assays for universal or canine-, bovine-, and human-specific targets, as well as a *Catellicoccus marimammalium* assay designed for gull fecal bacteria targets.[11,13,14] The universal markers consist of the Order *Bacteroidales*, primarily the genus *Prevotella* and *Bacteroides*. Additionally, fecal coliforms (FC) and *Enterococcus* spp. were quantified as conventional FIB of interest. All water and sediment samples were placed on ice in a cooler and immediately transported back to the AWWU lab within 1 hour for processing the FC and *Enterococcus* spp.

Water samples

Samples were collected 6 cm below the water surface into two 10 L, sterile, polyethylene containers using clean, plastic 480 mL cups to pour the water into the container, for a total volume of approximately 20 L. Each sediment sample was collected from benthic substrate at the same location as the matching water sample. Intertidal water samples were collected at a site just east of Point Woronzof (see Fig. 1) where the influence of a gyre on flood tide was reported and may be capable of transporting the effluent from the outfall site shoreward. The effluent samples were collected into sterile 250 mL polyethylene twist-top jars, from a faucet tapped into a well-mixed point downstream from the chlorination input in the effluent line, for a total of 10 L.[10]

All water and effluent samples were concentrated using an ultrafiltration process the same day as collection, or immediately the next morning (within 14 h) if collected late the previous day. This process concentrated the water sample from 20 L to

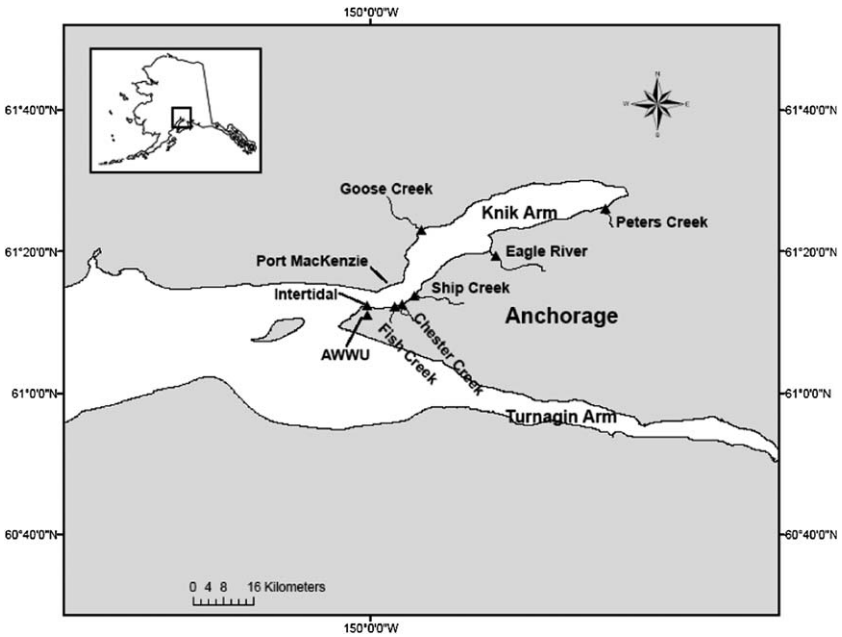

Fig. 1 Map of upper Cook Inlet showing sampling sites (solid black triangles) and location of Anchorage Water and Wastewater Utility (AWWU).

Table 1 Characteristics of environmental sampling sites in upper Cook Inlet, Alaska using salinity to define water type

Sample site	Location	Urban or rural	Water type
1	Peters Creek	Rural	Fresh
2	Eagle River	Rural	Estuarine
3	Ship Creek	Urban	Estuarine
4	Goose Creek	Rural	Estuarine
5	Chester Creek	Urban	Estuarine
6	Fish Creek	Urban	Estuarine
7	Intertidal zone	Urban	Marine
8	Effluent	Urban	Estuarine

approximately 250 mL using a Fresenius open-ended filtration system, which concentrates all microbe types simultaneously.[15] Negative control field blanks consisting of purified deionized water were run with each sample set. The retentate was shipped on ice to the University of California, Davis for target microbe detection.

Sediment samples

Sediment core samples were collected down to an approximate depth of 6 cm with sterile plastic 25 mL tubes at the site where water was also sampled. Each sediment core was processed for extraction of microbes based on a slight modification of treatment number one, as described in Boehm *et al.*,[12] which resulted in a simple extraction method that was reported to produce one of the highest fecal microbe recoveries. Briefly, the treatment consisted of measuring 10 g of sediment and placing it into a sterilized 250 mL polypropylene bottle, adding 60 mL of sterile, deionized water, and hand-shaking for two minutes over an arc of approximately 10 cm. After a 30 s settling time, the supernatant was decanted into a sterile whirl-pak bag by pouring off carefully to avoid including sand. Another 40 mL of deionized water was added to the sand, hand shaken again for two minutes (hand swirling was used in the original description), allowed to settle for 30 s and decanted into the same sterile bottle used after the first rinse. This resulted in 100 mL of supernatant (diluted 1 : 10) that was used to recover the same suite of microbes described for the water samples.

Pathogenic bacterial culture

Water and sediment supernatant samples were plated onto selective media based on established protocols.[16] For detection of *Salmonella* spp., samples were filter incubated for 24 h on xylose lysine desoxycholate (XLD) medium at 35.5 °C. A second filter was pre-enriched in selenite broth for 24 h at 37 °C, prior to subculturing 200 μL onto XLD agar.[17] Yellow to yellow-red colonies with black centers (H_2S-producers) were subcultured on 5% defibrinated sheep blood agar for final biochemical confirmation. Incubation of filters on thiosulfate-citrate-bile salt-sucrose (TCBS) agar (Hardy Diagnostics, Santa Maria, CA, U.S.A.) for 24 h at 35.5 °C was used to isolate *Vibrio* spp. A second filter was soaked in alkaline peptone water as enrichment broth, and after 24 h incubation at 37 °C, 200 μL was subcultured onto TCBS. Yellow and green colonies on TCBS were subcultured into 5% defibrinated sheep blood agar for biochemical confirmation followed by testing with API 20E strips (bioMérieux SA, l'Etoile, France) for further species verification.

Protozoal enumeration

Environmental samples were analyzed for *Cryptosporidium* oocysts and *Giardia* cysts using the U.S. Environmental Protection Agency (E.P.A.) method 1623 that involves filtration,

filter elution, centrifugation, and immunomagnetic separation (IMS) of parasites from resuspended pellets.[18] Briefly, water and sediment-derived samples were concentrated prior to IMS using centrifugation that produced a sediment pellet that was usually less than 0.5 mL. If the pellet volume was found to be greater than 0.5 mL, it was diluted with nanopure water so that each 5 mL of resuspended pellet was equivalent to 0.5 mL packed pellet volume. The IMS was performed twice with magnetic beads eluted with acid washes, and each wash (50 μL each) was transferred to a slide well containing sodium hydroxide to neutralize the pH. Direct fluorescent antibody testing was performed with the EasyStain kit (BTF, Sydney, New South Wales, Australia) per manufacturer's instructions, followed by individual *Cryptosporidium* oocyst and *Giardia* cyst counts on an epifluorescent microscope. Counts were reported as oocysts or cysts per 10 L of feed (original unconcentrated) sample. Negative controls consisting of purified deionized water were run with each sample set.

Enteric virus

A quantitative PCR (qPCR) assay was used to determine filtration recovery of norovirus genogroups GI and GII, and included a positive as well as negative control.[7] Water samples were concentrated as previously described, with additional concentration with polyethylene glycol 6000.[19] The High Pure viral nucleic acid kit (Roche Molecular Biochemicals Ltd, Mannheim, Germany) was used to extract viral RNA from the concentrates (200 μL), per manufacturer instructions. Reverse transcription (RT) was carried out using the Super-Script III first-strand synthesis system for RT-PCR (Invitrogen, Carlsbad, CA, U.S.A.) at 50 °C for 30 min, followed by an enzyme inactivation step of 95 °C for 4 min, and then held at 4 °C until real-time PCR amplification was performed.

Fecal host source markers

Quantitative PCR (qPCR) assays were used to identify host-associated 16S rRNA gene markers of the Order *Bacteroidales* for canine-, bovine-, human-specific and general (universal) markers.[13,20,21] The avian target was *C. marimammalium*.[14] The surrogate *Acinetobacter baylyi* ADP1 was added to water and sediment samples before ultrafiltration. Recoveries from the filtration process were calculated by measuring *A. baylyi* concentrations in subsamples of pre- and post-filtration samples. A resulting volume of 50 mL from the filtration process was subjected to DNA extraction and qPCR quantification as described in Schriewer *et al.*[11] Each negative control was also spiked with *Acinetobacter*, allowing for estimates of recovery during sample processing for qPCR measurements.

Fecal coliform and *Enterococcus* enumeration

Fecal coliforms were detected using multiple tube fermentation method SM 9221E.[22] The first procedure stage estimated total coliforms and the second stage estimated fecal coliforms. Any positive tubes went on to fecal coliform detection using EC confirmation medium (Difco®, Becton Dickinson and Company, Franklin Lakes, New Jersey, U.S.A.). The EC tubes were incubated at 44 ± 0.2 °C for 24 ± 2 h. Tubes with gas production were again considered positive. Results were obtained from the most probable number (MPN) table based on the number of positive tubes in each dilution.

Enumeration of enterococci in water and sediment samples was performed using the Enterolert test kit (IDEXX Laboratories, Westbrook, ME, U.S.A.).[23] Briefly, 90 mL volumes of sterile deionized water were added to IDEXX's dehydrated media in the 100 mL sterile jars supplied, followed by hand-shaking 2–3 times over 5 min to dissolve the media. To this solution was added 10 mL of the target water or diluted sediment supernatant sample volumes followed by shaking. The contents of the jars were poured into sterile Quanti-Tray 2000 trays and heat sealed. Quanti-Trays were incubated according to manufacturer's instructions at 41 ± 0.5 °C for 24 h. Marine and estuarine water samples were tested at a dilution of 1 : 10 to minimize false positive results due to marine *Vibrio* spp.[24] For each set of bacterial tests, a negative control water sample (Milli-Q water, Millipore Corporation, Billerica, MA, U.S.A.) was also run.

Environmental risk factors

Multiple environmental variables were evaluated as risk factors associated with fecal microbe detection. Risk factors included matrix (water *versus* sediment), water type (fresh *versus* estuarine/marine, which was based on measured chloride concentration [mg dL^{-1}]), water temperature (≥ *versus* <13 °C), recent precipitation (present *versus* absent for preceding seven days), and collection month (June [late spring] *versus* August [late summer]). Marine and estuarine waters were combined due to small sample size and other correlating factors. Sample location was defined as urban or rural based on 2010 human population figures for the census area associated with the sample site (http://factfinder.census.gov).

Data analyses

Prevalence was determined for each microbe, and results for *Cryptosporidium* and *Giardia* were pooled for the bivariate and multiple regression analyses due to sparse data. The Wilcoxon rank sum test was used to test for differences in precipitation (city of Anchorage) and streamflow (Ship Creek) between the months of June and August, 7 and 30 days prior to the start of sample collection, as well as for evaluating differences in *Enterococcus* and fecal coliform counts by month and matrix.[25] Simple logistic regression analyses, followed by multiple logistic regression models, were used to quantify the strength of association between environmental variables and detection of the microbe group of interest that included presence or absence of *Vibrio* spp., protozoa, universal *Bacteroidales* marker, and human-specific *Bacteroidales* marker. Model building was performed by including hypothesized risk factors and adding potential confounding variables with evidence for association ($p < 0.20$) in the univariable analyses.[26] Exact logistic regression was used to evaluate the association between the putative risk factors and the probability of a sample being positive.

Percent recovery for the IMS-DFA testing for protozoa was calculated by dividing the number of oocysts counted on the

DFA slide by the number of (oo)cysts expected per test aliquot.[9] The expected number of (oo)cysts per test aliquot was determined by multiplying the number of (oo)cysts spiked into the sample by the proportion of matrix analyzed.

The true concentrations of fecal host-associated genetic markers were estimated with a previously validated statistical model based on the Law of Total Probability. The model used the Monte Carlo (MC) method to predict the concentration of each marker type from raw concentrations, resulting in host-associated output distributions.[27] The initial concentration of each host-associated marker was incorporated into the model. Distributions of the probabilities of obtaining false information (referring both to false positive and false negative findings) were estimated, and along with the measurement error derived from the qPCR reactions, were then sampled by MC methods. The resulting set of equations given by the Law of Total Probability allowed for estimation of the true concentration of each marker in a sample, or the contribution of each host to the total *Bacteroidales* signal. All data analyses were performed using Stata version 11.0 (Stata Corp., U.S.A., 2012), except the Monte Carlo modeling that was performed with R (The R Project for Statistical Computing; http://www.r-project.org/). *P*-values <0.05 were considered significant.

Results

Water and sediment were sampled from eight sites in upper CI, including rural and urban locations, ranging south from Peters Creek in the northeast to the western edge of Anchorage (Table 1) (Fig. 1). The prevalence of microbes by sample location and matrix is shown in Table 2 for the total of 41 samples collected, 23 in early June and 18 during the last week of August. *Salmonella* was not detected at any of the sites. For both months, *Vibrio* spp., specifically *V. alginolyticus* and *V. fluvialis*, were the fecal microbes detected most often. The prevalence of *Vibrio* was higher in sediment compared to water samples. Chester and Fish Creeks had the highest prevalence of *Vibrio* compared to other sites. Assay limits of detection for cultured bacteria were 5 cfu per L surface water based on matrix spiking studies.

Giardia prevalence was greater than that of *Cryptosporidium*, both overall and by matrix type, and *Cryptosporidium* was not detected in any sediment samples. *Cryptosporidium* and *Giardia* spp. were detected at low levels during both months. Concentrations for the former were lower than the latter, and for both organisms were higher in water compared to sediment. *Cryptosporidium* and *Giardia* concentrations in surface water ranged from 0–94 and 0–23 oocysts per 10 L sample feed, respectively, while in effluent, the ranges were 0–28 and 0–14 oocysts per 10 L, respectively. The range of *Giardia* in sediment was 0–8 cysts per 10 g of sample. For IMS-DFA analysis of surface water samples spiked with 300 (oo)cysts ($n = 5$), mean% recoveries for oocysts and cysts were 35% and 24% (range = 10–57% and 40–55%), respectively. For spiked sediment samples ($n = 3$), mean percent (%) recoveries for *Cryptosporidium* and *Giardia* were 27 and 33%, with ranges of 7–52% and 8–75%, respectively. Using the mean recovery data in water and sediment, the true number of (oo)cysts present in an environmental sample was estimated to be approximately three times higher than that visualized using the IMS-DFA method. Assay limits of detection were 5

Table 2 Prevalence of fecal bacteria, protozoa, and viruses in water and sediment samples by matrix and site in upper Cook Inlet, Alaska (2011)

	Prevalence (%) of target microbes						
						Norovirus[b]	
Matrix/sites	n^a	*Salmonella* spp.	*Vibrio* spp.	*Cryptosporidium* spp.	*Giardia* spp.	NVGI	NVGII
Water							
Peters Creek	2	0	0	100	100	50	0
Eagle River	2	0	50	0	100	50	0
Ship Creek	3	0	0	67	67	0	0
Goose Creek	2	0	0	50	50	0	0
Chester Creek	3	0	67	33	33	50(2)[a]	0
Fish Creek	3	0	100	0	33	50(2)	0
Intertidal	4	0	75	50	50	50(2)	0
Effluent	4	0	0	100	100	50(2)	50(2)
Water prevalence		0	39	52	65	35	6
Sediment							
Peters Creek	2	0	0	0	0	0(1)	0(1)
Eagle River	2	0	50	0	0	0(1)	0(1)
Ship Creek	2	0	0	0	0	0(1)	0(1)
Goose Creek	2	0	100	0	0	0	0
Chester Creek	3	0	100	0	67	0(0)	0(0)
Fish Creek	3	0	67	0	67	0(2)	0(2)
Intertidal zone	4	0	25	0	0	0(0)	0(0)
Sediment prevalence		0	50	0	22	0	0
Overall Prevalence		0	44	29	46	25	4

[a] Number of samples tested (except norovirus, where number tested is in parentheses). [b] NVGI = Norovirus Group I; NVGII = Norovirus Group II.

parasites per L surface water and 10 parasites per g sediment based on matrix spiking studies.

Norovirus Group I was detected more frequently in June and more commonly than norovirus Group II, but was not detected in any sediment samples. The range of Group I concentration over both months was 10.7–88.6 gc mL^{-1} of water sample. Norovirus Group II was detected in one sample (6%) of water that was collected in June, but was not detected in any sediment samples. The concentration for that sample was 252 gc mL^{-1}. The assay limits of detection (ALOD) for NVGI and NVGII were 0.2 gc μl^{-1} and 0.3 gc μl^{-1}, respectively.

Overall, the most frequently detected fecal host-associated marker was human source, followed by bovine (Table 3). In water matrices, human-associated signal was the most prevalent, followed by canine-associated markers. In sediment, the prevalence was again highest for human-associated markers followed by equal prevalences of bovine- and avian-associated markers, the latter of which was not detected in water. The greatest diversity of marker types occurred in the effluent (bovine, canine, and human) and in Goose Creek sediment (avian, bovine, and human). The marker-specific ALODs were relatively low, as follows: 6.1, 0.3, 4.8, and 2.4 gc mL^{-1} (canine, livestock, human and universal markers, respectively).

Monte Carlo methods allowed for further analysis of samples in which *Bacteroidales* markers were initially detected for canine, bovine, and human species in water and sediment samples. The MC model produced an estimate of the 'true concentration' of *Bacteroidales* for each host-associated group in each sample and helped to correct intrinsic false information associated with qPCR assays. The model results for each tested sample showed a lack of detection for some of the host-associated markers (Table 3), and highlight that the host markers detected were generally at low levels compared to the 'universal' marker that represents total fecal load in that sample. For example, canine and bovine markers in water samples were no longer reported in the MC output due to their low concentrations relative to total universal *Bacteroidales* signal that represents all contributing host sources. Human markers in water samples remained at detectable levels.

Notable differences between FC and *Enterococcus* counts occurred by matrix type, sampling month, location, and recent precipitation (Fig. 2). Fecal coliform counts (and corresponding ranges) were greater than for *Enterococcus*, when compared by recent precipitation, and sampling month and matrix type. Counts in sediment samples were frequently 10–100 times greater than in water in Chester and Peters Creeks. Fecal coliform counts were significantly higher in sediment than water ($Z = -3.357$, $p < 0.001$), while non-significantly greater *Enterococcus* counts were observed in sediment compared to water. Mean precipitation and streamflow were significantly greater in August than in June, when both environmental factors were examined 30 days prior to sample collection ($Z = -3.297$, $p = 0.001$ and $Z = -3.918$, $p < 0.001$, respectively). *Enterococcus* counts (MPN per 100 mL) were similarly highest in the urban creeks and effluent (298 MPN per 100 mL), and also increased in the August samples with the exception of the effluent counts that decreased to levels of <20 MPN per 100 mL. Significant differences in *Enterococcus* and FC counts were not noted for sample month and location (Fig. 2). Two sediment samples in August had relatively high counts, including Ship Creek (325 per 100 mL) and Fish Creek (475 per 100 mL), but sediment quality standards have not been established. The three outliers noted

Table 3 Detection of *Bacteroidales* host-associated marker in water and sediment samples by site and month, in upper Cook Inlet, Alaska (2011), with relative contribution (prevalence) of each host source to total marker concentration by month (June/August)

		Detection of fecal host source markers[b]				Percent (%) contribution to total fecal host signal by month			
Matrix/sites	n^a	Canine	Livestock	Human	Avian	Canine	Livestock	Human	Avian
Water									
Peters Creek	2	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Eagle River	2	0/0	0/0	1/0	0/0	0/0	0/0	0.10/0	0/0
Ship Creek	2	0/0	0/0	0/1	0/0	0/0	0/0	0/0.02	0/0
Goose Creek	2	0/0	0/0	1/0	0/0	0/0	0/0	0.5/0	0/0
Chester Creek	2	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Fish Creek	2	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Intertidal zone	2	0/1	0/0	1/1	0/0	0/0	0/0	1.5/0.4	0/0
Effluent	2	1/1	1/1	1/1	0/0	0/0	0/0	13.4/34.2	0/0
Overall water %		19	13	44	0				
Sediment									
Goose Creek	2	0/0	1/0	1/1	1/0	0/0	0/0	0/0	0/0
Fish Creek	2	0/0	0/0	1/0	1/0	0/0	0/0	0/0	0/0
Peters Creek	1	—/0	—/0	—/0	—/0	—/0	—/0	—/0	—/0
Eagle River	1	—/0	—/0	—/0	—/0	—/0	—/0	—/0	—/0
Ship Creek	1	—/0	—/1	—/1	—/0	—/0	—/0	—/0	—/0
Overall sediment %		0	29	57	29				
Overall %		13	17	48	9				

[a] Number of samples tested at each site. [b] 1 = detected, 0 = not detected, — = not tested.

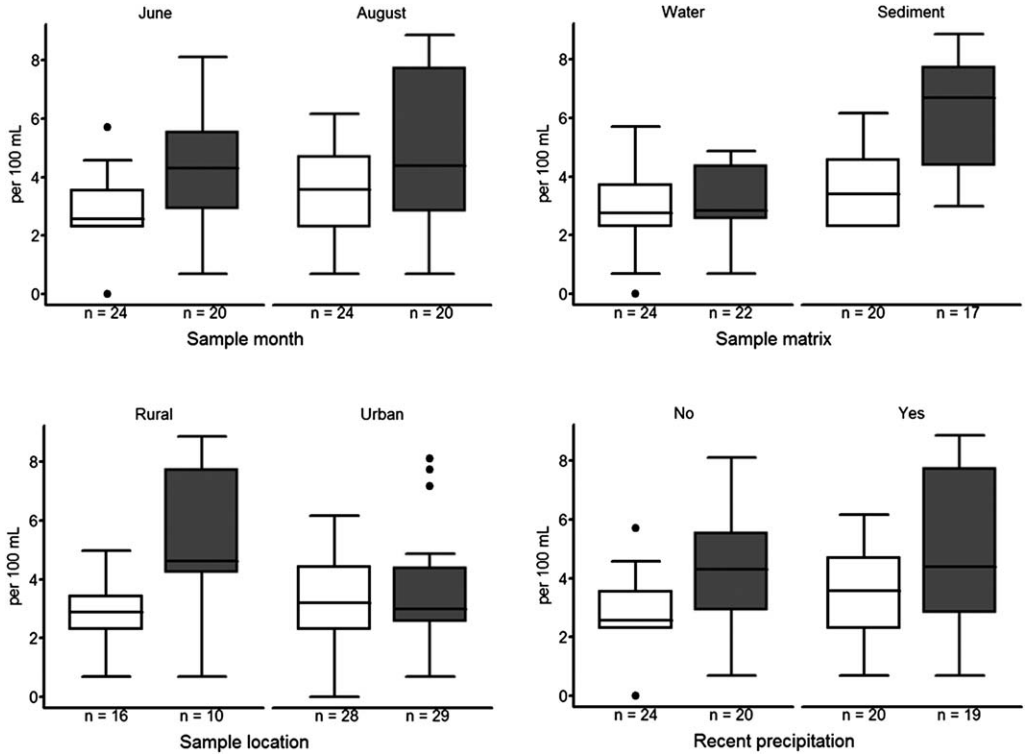

Fig. 2 Log-transformed ranges of *Enterococcus* counts (most probable number per 100 mL) (white) and fecal coliform counts (colony forming units per 100 mL) (dark gray) in water and sediment samples collected in upper Cook Inlet, Alaska by month, matrix, location type, and recent precipitation.

for urban FC counts were all samples obtained in August and represented creeks that are heavily utilized by humans, domestic dogs, and wildfowl (Ship, Chester, and Fish Creeks). Their outflow sites could expose belugas utilizing the nearshore waters to relatively high concentrations of potential pathogens compared to other waterways. Binary logistic regression was

Table 4 Results of simple logistic regression models for detection of fecal pathogens in water and sediment from upper Cook Inlet, Alaska

	Vibrio spp.		Any target fecal protozoa		Human *Bacteroidales* marker		Any host-associated marker	
Risk factors[a]	OR	*P* value	OR	*P* value	OR	*P* value	OR	*P* value
Month								
August (June)	1.6	0.6	1.2	1.0	0.4	0.5	0.2	0.1[b]
Sample location								
Rural (Urban)	2.4	0.3	1.2	1.0	1.7	0.8	1.6	0.9
Matrix								
Sediment (Water)	1.4	0.8	0.1	0.002[b]	1.7	0.9	1.0	1.0
Water type								
Estuarine/marine (Freshwater)	1.9	0.7	0.9	1.0	1.5	1.0	1.9	0.9
Water temperature ≥13 °C								
Yes (No)	3.9	0.3	0.5	0.7	2.5	0.7	1.2	1.0
Precipitation in past week								
Yes (No)	2.0	0.4	0.9	1.0	0.4	0.5	0.2	0.1[b]

[a] Referent categories in parentheses beside corresponding risk factor. OR, odds ratio. [b] *P* value ≤0.20 was considered for inclusion in the multivariate model.

used to assess the association between the indicator organisms, fecal coliforms and *Enterococcus* spp., and the presence/absence of *Cryptosporidium*, *Giardia*, and *Vibrio*, but no significant associations were detected. Simple logistic regression was used to evaluate the strength of association between individual environmental variables and a sample testing positive for each type of microbe group. The odds ratio (OR) and associated *P* values for factors significantly associated with detection of *Vibrio*, any protozoa, human-specific *Bacteroidales* marker, or any host-associated marker are presented in Table 4. The other microbial groups were not included, either due to the sparse nature of the data (*Salmonella* and norovirus), or if the target was detected in almost every sample (fecal coliforms). The presence of *Vibrio* and human *Bacteroidales* marker were not significantly associated with any of the environmental variables based on univariable analyses. The detection of any *Bacteroidales* marker in a sample was independently associated with sample month (OR = 0.2, $p = 0.1$) and recent precipitation (OR = 0.2, $p = 0.1$), but neither remained significant when both factors were analyzed together in a multivariable model due to high collinearity with each other (95%). Detection of any protozoal organism in sediment was ten times less likely (OR = 0.1) than in water ($p = 0.002$). Water temperature and sample location were not significantly associated with the presence of any of the microbe groups.

Discussion

This study used a novel approach to demonstrate the presence of potentially pathogenic bacteria, protozoa, and viruses in water and sediment samples from regions in and around the municipality of Anchorage in the upper Cook Inlet, Alaska watershed, along with using host-associated fecal markers to track fecal sources at multiple sites. Overall, a greater concentration of fecal loading from human-specific marker was identified relative to the other host-associated assays, with the majority of total fecal load remaining uncharacterized, likely due to wildlife contributions for which targeted assays are not yet available. The study findings highlight the role microbial source tracking may play in supplementing water quality investigations of pathogen pollution into coastal aquatic environments. The specific findings of this study are most relevant to the Cook Inlet ecosystem, and may have limited generalizability to other geographic areas, but the water quality testing approach can be tailored to meet the needs of many multi-use ecosystem settings.

The two types of *Vibrio* spp. that were detected in this study were primarily in samples from urban sites. *Vibrio fluvialis* is a potentially pathogenic species that is reported to cause sporadic infections and outbreaks of diarrhea in humans,[28] and it has been isolated from marine and estuarine environments.[29] *Vibrio alginolyticus*, the most widespread environmental species, is commonly non-pathogenic but can cause gastroenteritis or wound infections under favorable conditions.[30] Lack of detection of *Salmonella* spp. could have been due to absence or to one or more reasons such as damage to the bacterial cells from surrounding conditions, inhibition from competing bacteria during culture, the matrix interfering with detection of the bacteria, environmental temperatures, rainfall pattern, or the bacteria remaining in a metabolically viable but nonculturable state.[31,32]

Noroviruses, members of the *Caliciviridae* family, are found in many mammalian species, including humans, pigs, cattle, and mice. They are subdivided into five genogroups (GI–GV), of which GI and GII contain most of the strains relevant to human disease.[34] In this study, norovirus GI was more prevalent than GII, both were within ranges previously recorded, and both were more commonly detected in residential and mixed use water than in urban waters (W. Miller and S. Wuertz, unpubl. work). Norovirus infection in humans is a major cause of non-bacterial gastroenteritis worldwide, most commonly associated with food- and waterborne outbreaks that occur *via* the fecal–oral route, with a recent study also suggesting the role of pets in transmission of the virus to humans.[33,34] The relative lack of GII detection may be due to this particular strain escaping detection by the available primers for the RT-PCR assay, or due to low prevalence. Recently developed molecular techniques may help with future norovirus detection in water and sediment as an alternative fecal bioindicator to FC.[35,36]

A highly significant increase in detection of protozoal organisms was observed in water samples compared to sediment. This may be because the survival of *Giardia* has been shown to be adversely affected by the presence of sediment, possibly due to biological antagonism from other microorganisms or the presence of organic substances.[37] Alternatively, the adherence of the protozoa to the sediment particles may have resulted in reduced detection of the organisms in the sediment supernatant compared to water, although the sediment processing protocol we used has been successfully used to detect protozoa in supernatant in other studies.[12] *Giardia*, which was detected more frequently than *Cryptosporidium*, had a higher prevalence during June sampling in both water and sediment samples, in contrast to the other microbes that were detected more frequently in August. Although a significant proportion of both cysts and oocysts readily attach to organic biological particles in their environment such as clay, sand, plankton, and algae,[38] the greater freshwater flow rates in August may have washed away much of the biological particles to which the (oo) cysts were attached. In view of the variable efficiency of detection by the methods used for monitoring *Cryptosporidium* and *Giardia*, parasite recovery efficiency was evaluated in both sample matrices.[3] Percent recovery in water was greater for *Cryptosporidium* than for *Giardia*, while the converse was true in sediment, with the exception of Chester Creek. Low (oo)cyst recovery remains a problem in water quality monitoring methods, especially in waters containing high turbidity or total suspended solids.[38] Possible explanations for these variations include variable (oo)cyst loss during processing of the sediment samples, varying sediment types, and background matrix of the sample affecting (oo)cyst visualization.[39,40]

No significant associations were observed between target fecal microbes and samples collected in warmer water temperatures or estuarine waters, nor in urban locations when assessed in logistic regression models. Increased detection of

universal *Bacteroidales* markers after recent precipitation was associated with August sampling, the month during which the annual mean rainfall is highest in the Anchorage area (http://pafc.arh.noaa.gov/climate/index.php). The prevalence of *Bacteroidales* recovered from the sediments at multiple sites during this study was higher than that in water, which may reflect the previously described persistence of other fecal microbes in sediments.[41] The presence of human-specific marker in so many samples may represent loading of human waste through leakage of aged septic systems, deposition of waste from outdoor activities such as camping or hiking, stormwater runoff, or could be related to the assay specificities and sensitivities in this study. The differences noted between marker prevalence and true concentrations from the MC model (Table 3) demonstrate that the majority of fecal contribution cannot be attributed to a host source, so additional host marker development will help to better characterize fecal pollution.

There are several advantages to using the MC model to estimate true concentrations of host-associated *Bacteroidales* markers. First, from true concentrations estimated by the model, expected values, confidence intervals, and other statistical characteristics can be easily evaluated. The distributions of true concentrations may be further input into quantitative microbial risk assessments, total maximum daily load (TMDL) determinations, and other models in order to provide valuable methods to evaluate and implement microbial water quality management programs. The information can subsequently be used to infer the potential health risk due to pathogens that may be associated with the corresponding host feces and to help elucidate the sources of host-associated fecal contribution to an aquatic ecosystem.

A limitation of the model is the inability to completely account for false positive and negative information intrinsically associated with qPCR assays.[27] The lack of detection of bovine-, canine-, and some human-specific *Bacteroidales* genetic markers after analysis by the MC model may be due to several factors. First, the presence and abundance of target genetic markers for an animal host may be variable from sample to sample for a specific host. Second, false positive findings may result from a primer for one specific species host amplifying *Bacteroidales* DNA from another host. For example, bovine markers were detected in four samples (Table 3), some in urban locations (Fig. 1), suggesting possible cross-reaction from feces deposited by moose (*Alces alces*) living in and around Anchorage.[42] Third, *Bacteroidales* DNA for a specific host may not be detected by the primer designed for that host (loss of specificity). Finally, measurement errors may occur during sample preparation and processing.[27] Lastly, while it is recognized that the small sample size may preclude making definitive conclusions about study results, this project provides an initial assessment of the presence of fecal pathogens, indicator bacteria, and novel host specific marker tracking in water and sediment samples from a geographic area that is relatively remote but urbanized. Future monitoring efforts should include increasing the sample volume size to allow for greater quantitative evaluation of fecal pathogen presence using more than one type of methodology. Future studies will also increase the sample size and allow for more thorough evaluation of fecal pathogen dynamics in coastal ecosystems.

As expected, FC were recovered from almost every sample. The counts at most sites were markedly greater in August, with some counts increasing more than 10-fold subsequent to the heavier rainfall in August that also coincided with the increased river and streamflows noted during sample collections (Fig. 2). The FC concentrations in the effluent were relatively low, and were within a range commonly recovered from previous sampling studies.[10] Counts for FC (Fig. 2) did not exceed acceptable limits based on Alaska's state water quality standards for drinking (freshwater) and marine recreational waters (20 FC mL^{-1} and 200 FC mL^{-1}, respectively).[43] *Enterococcus* counts did exceed the federal and state single-sample limit (276 enterococci per 100 mL) in a June effluent sample (298 per 100 mL).

The lack of association between fecal indicators and pathogen presence underscores the failure of single indicator organisms to adequately predict fecal pathogens or health outcomes associated with pathogen contact in water and sediment. This suggests that monitoring a suite of indicator organisms is more likely to be predictive of pathogen presence rather than reliance on a single indicator.[44,45] The detection and concentration of indicator organisms may also not correlate well with exposure to pathogens and health outcomes due to factors such as virulence of the pathogen, exposure dose, and variation in immunologic response of the exposed individual.[46]

The influence of temperature and recent precipitation should be considered when interpreting the results of microbe detection. In CI, spring freshwater river and stream temperatures are strongly influenced by snowmelt and runoff. Though snowmelt is expected to increase river and streamflow during spring and early summer, freshwater flow was actually greater in August due to heavy rainfall. Temperatures at the end of summer/beginning of autumn are generally higher than spring/early summer due to the relative lack of snow, less streamflow, and, during late autumn, the release of latent heat from formations of ice.[47]

Like many populated areas in other parts of the United States and around the world, the main causes of coastal water pollution in Alaska are thought to be urban and agricultural/livestock runoff, wastewater, and natural resource utilization such as mining and logging.[28] Non-point and point sources, including wastewater treatment plant effluent and stormwater runoff, transport pathogens to the marine environment. Microorganisms have incomplete removal during wastewater treatment, thus posing health risks to humans and animals in contact with effluent. For instance, in our study pathogens were commonly detected in effluent and in other studies bacterial pathogens such as *Salmonella* and *Vibrio* have been detected during all stages of sewage treatment and even in chlorinated effluent.[48,49]

The relatively small number of positive results compared to non-detects, in this study, does not mean that pathogens are absent in this ecosystem given the inherent variability in environmental dynamics and detection methodologies. Exposure of belugas to terrestrial-source fecal pathogens must be considered possible through consumption of seawater and sediment

during foraging, as well as through exposure of superficial skin wounds and abrasions to water and sediment. Exposure to pathogens in river mouths such as Eagle River, where belugas often congregate and socialize, is still a consideration.[50] *Vibrio* spp. are documented to cause disease in marine mammals,[51] while the risk of disease due to Norovirus is unknown; however, with detection of the virus in several water samples of varying salinities, it seems that belugas may be exposed during travel within in the Inlet or when congregating in estuarine areas such as Eagle River where the presence of human and canine fecal pathogens were detected. Protozoal organisms such as *Giardia* have a relatively high prevalence here as elsewhere, though their significance to the health of belugas is unknown.[52] The results of this study suggest that fecal pathogens could pose a threat to belugas, and that further efforts should be directed toward repeated sampling of water and sediment over time to evaluate trends.

As Alaska's population grows and the natural resource-based economy expands in the Anchorage area, an increasing number of its waters, especially in urban areas, will face the threat of degradation. This study demonstrated that fecal pathogens detected in various matrices and locations appear to derive from both human and animal sources. Mitigation strategies may include identifying sources of runoff, leaking septic systems, areas of heavy dog use, working with local communities, industry stakeholders, and water utility personnel to identify techniques to further minimize waste deposition into bodies of water. Runoff from land-based pollution sources may stress the nearshore ecosystem and increasingly lead to human health concerns. Waterborne infections may threaten not only public health, but also place coastal economies at significant risk.

Conclusions

- *Giardia and Vibrio* spp. were the most commonly detected organisms in environmental matrices.
- Other than *Vibrio* bacteria, pathogens were more commonly detected in water samples compared to sediment samples.
- In water and sediment samples, the most frequently detected fecal host-associated marker was human source.
- The findings of 'recent precipitation' and 'sediment' as environmental factors associated with detecting universal *Bacteroidales* marker and fecal protozoa, respectively, suggest that exposure of humans and marine life such as belugas to fecal pollution may be impacted by climatic and anthropogenic factors in the CI coastal ecosystem, and in other similar watersheds.
- While exposure of belugas to terrestrial-source fecal pathogens from effluent discharge may be relatively infrequent due to dilution of the effluent in the inlet from large tidal currents, the study findings may represent an underestimation of the presence of potential pathogens to which belugas are exposed.
- Future monitoring efforts in this region should include repeated sampling of water and sediment over time to evaluate trends in pathogen concentration at various locations, and with multiple detection methodologies considered for sensitive and specific pathogen detection.

Acknowledgements

The authors thank Craig Woolard, Brett Jokela, Robert Gustafson, Paul Smith, Steve Gaither, Mark Spano, Andrew Johnson, Melissa Harrower, and the rest of the Alaska Water and Wastewater Utility personnel for use of their laboratory space to process samples, their expertise and knowledge in water quality testing, and logistical support. Christopher Garner from Joint Base Elmendorf-Richardson and Howard Golden and Todd Rinaldi from the Alaska Department of Fish and Game kindly provided boat support and collected the Goose Creek samples. The authors also thank Terra Berardi, Michael Maki, and Alexander Schriewer of UC Davis for their expertise and assistance. This work was supported by NOAA Fisheries contract HA133F-10-SE-3662.

References

1 *Cook Inlet Basin ecoregional assessment*, Nature Conservancy of Alaska, Anchorage, 2003, http://east.tnc.org/east-file/71/Cook_Inlet_ Ecoregional_Assessment.pdf.
2 *Basic Counts/Population*, U.S. Census Bureau, Alaska, 2011, American Factfinder, http://www.census.gov/, accessed April 2011.
3 H. V. Smith and A. M. Grimason, in *Handbook of Water and Wastewater Microbiology*, ed. D. Mara and N. Horan, Academic Press, London, 2003, ch. 40, pp. 695–756.
4 *2008 Status Review and Extinction Risk Assessment of Cook Inlet Belugas (*Delphinapterus leucas*)*, Alaska Fisheries Science Center, NOAA, NMFS, AFSC Processed Rep. 2008-02, Seattle, 2008, http://www.afsc.noaa.gov/Publications/ProcRpt/PR%202008-02.pdf, accessed October 2011.
5 R. Higgins, *Can. Vet. J.*, 2000, **41**, 105–116.
6 J. M. Hughes-Hanks, L. G. Rickard, C. Panuska, J. R. Saucier, T. M. O'Hara, L. Dehn and R. M. Rolland, *J. Parasitol.*, 2005, **91**, 1225–1228.
7 S. Wolf, W. M. Williamson, J. Hewitt, M. Rivera-Aban, S. Lin, A. Ball, P. Scholes and G. E. Greening, *Appl. Environ. Microbiol.*, 2007, **73**, 5464–5470.
8 J. T. Lisle, J. J. Smith, D. D. Edwards and G. A. McFeters, *Appl. Environ. Microbiol.*, 2004, **70**, 7269–7276.
9 W. A. Miller, M. A. Miller, I. A. Gardner, E. R. Atwill, B. A. Byrne, S. Jang, M. Harris, J. Ames, D. Jessup, D. Paradies, K. Worcester, A. Melli and P. A. Conrad, *Microb. Ecol.*, 2006, **52**, 198–206.
10 *Monitoring Program Annual Report, January-December 2010*, Anchorage Water and Wastewater Utility, Report prepared by Kinnetic Laboratories Incorporated, Anchorage, 2011, http://www.awwu.biz/eInfo/Files/Cook%20Inlet%20Water%20Quality/2010%20-%20Asplund%20Wastewater%20Treatment%20Facility %20Annual%20Monitoring%20Report.pdf.
11 A. Schriewer, W. A. Miller, B. A. Byrne, M. A. Miller, S. Oates, P. A. Conrad, D. Hardin, H.-H. Yang, N. Chouicha, A. Mellik,

D. Jessup, C. Dominik and S. Wuertz, *Appl. Environ. Microbiol.*, 2010, **76**, 5802–5814.
12 A. B. Boehm, J. Griffith, C. McGee, T. A. Edge, H. M. Solo-Gabriele, R. Whiteman, Y. Cao, M. Getrich, J. A. Jay, D. Ferguson, K. D. Goodwin, C. M. Lee, M. Madison and S. B. Weisberg, *J. Appl. Microbiol.*, 2009, **107**, 1740–1750.
13 B. J. Kildare, C. M. Leutenegger, B. S. McSwain, D. G. Bambic, V. B. Rajal and S. Wuertz, *Water Res.*, 2007, **41**, 3701–3715.
14 J. Lu, J. W. Santo Domingo, R. Lamendella, T. Edge and S. Hill, *Appl. Environ. Microbiol.*, 2008, **74**, 3969–3976.
15 V. B. Rajal, B. S. McSwain, D. E. Thompson, C. M. Leutenegger, B. J. Kildare and S. Wuertz, *Water Res.*, 2007, **41**, 1411–1422.
16 E. J. Baron and R. B. Thomson Jr, in *Manual of Clinical Microbiology*, ed. J. Versalovic, K. C. Carroll, G. Funke, J. H. Jorgensen, M. L. Landry and D. W. Warnock, ASM Press, Academic Press, 10th edn, 2011, ch. 16, pp. 286–330.
17 USEPA, *Method 1682: Salmonella in sewage sludge (biosolids) by modified semisolid Rappaport-Vassiliadis (MRSV) medium, EPA-821-R-06-14*, Office of Water, Washington DC, 2006.
18 USEPA, *Method 1623: Cryptosporidium and Giardia in water by Filtration/IMS/IFA,EPA-815-R-05-002*, Office of Water, Cincinnati, OH, 2005.
19 V. R. Hill, A. L. Polaczyk, D. Hahn, J. Narayanan, T. L. Cromeans, J. M. Roberts and J. E. Amburgey, *Appl. Environ. Microbiol.*, 2010, **71**, 6878–6884.
20 A. E. Bernhard and K. G. Field, *Appl. Environ. Microbiol.*, 2000, **66**, 1587–1594.
21 L. K. Dick, A. E. Bernhard, T. J. Brodeur, J. W. Santo Domingo, J. M. Simpson, S. P. Walters and K. G. Field, *Appl. Environ. Microbiol.*, 2010, **71**, 3184–3191.
22 L. S. Clescer, A. E. Greenberg and A. D. Eaton, *Standard Methods for the Examination of Water and Wasterwater*, American Public Health Association, Washington DC, 2005, pp. 9–54.
23 G. E. Budnick, R. T. Howard and D. R. Mayo, *Appl. Environ. Microbiol.*, 1996, **62**, 3881–3884.
24 J. M. Pisciotta, D. F. Rath, P. A. Stanke, D. M. Flanery and V. J. Harwood, *Appl. Environ. Microbiol.*, 2002, **68**, 539–544.
25 *Real-time water data for the nation*, U.S. Geological Survey, Alaska, 2012, http://waterdata.usgs.gov/ak/nwis/rt, accessed January 2012.
26 D. W. Hosmer and S. Lemeshow, *Applied Logistic Regression*, Wiley, New York NY, 2nd edn, 2000.
27 D. Wang, S. S. Silkie, K. L. Nelson and S. Wuertz, *Water Res.*, 2010, **44**, 4760–4775.
28 C. H. Lai, C. K. Hwang, C. Chin, H. H. Lin, W. W. Wong and C. Y. Liu, *J. Infect.*, 2006, **52**, 95–98.
29 D. E. Lockwood, A. S. Kreger and S. H. Richardson, *Infect. Immun.*, 1982, **35**, 702–708.
30 S. D. Holmbert, *Infect. Dis. Clin.*, 1988, **2**, 655–676.
31 B. J. Haley, D. J. Cole and E. K. Lipp, *Appl. Environ. Microbiol.*, 2011, **75**, 1248–1255.
32 J. W. Santo Domingo, S. Harmon and J. Bennett, *Curr. Microbiol.*, 2000, **40**, 409–417.
33 M. M. Patel, A. J. Hall, J. Vinjé and U. D. Parashar, *J. Clin. Virol.*, 2009, **44**, 1–8.
34 M. Summa, C.-H. von Bondsdorff and L. Maunula, *J. Clin. Virol.*, 2012, **53**, 244–247.
35 T. Miura, Y. Masago, D. Sano and T. Omura, *Appl. Environ. Microbiol.*, 2011, **77**, 3975–3981.
36 H.-I. Tong, C. Connell, A. B. Boehm and Y. Lu, *Water Res.*, 2011, **45**, 5837–5848.
37 M. R. Karim, F. D. Manshadi, M. M. Karpiscak and C. P. Gerba, *Water Res.*, 2008, **38**, 1831–1837.
38 G. J. Medema, F. M. Schets, P. F. M. Teunis and A. H. Havelaar, *Appl. Environ. Microbiol.*, 1998, **64**, 4460–4466.
39 C. L. DiGiorgio, D. A. Gonzalez and C. C. Huitt, *Appl. Environ. Microbiol.*, 2002, **68**, 5952–5955.
40 L.-A. H. Krometis, G. W. Characklis and M. D. Sobsey, *Appl. Environ. Microbiol.*, 2009, **75**, 6619–6621.
41 B. D. Badgley, B. S. Nayak and V. J. Harwood, *Water Res.*, 2010, **44**, 5857–5866.
42 *Living with Moose*, Alaska Department of Fish and Game, 2011, http://www.adfg.alaska.gov/index.cfm?adfg=livewith.moose, accessed January 2012.
43 *Water Quality Standards – 18 AAC 70. Amended as of May 26, 2011*, Alaska Department of Environmental Conservation, 2011, http://www.dec.state.ak.us/water/wqsar/wqs/index.htm, accessed December 2011.
44 K. G. Field and M. Samadpour, *Water Res.*, 2007, **41**, 3517–3538.
45 V. J. Harwood, A. D. Levine, T. M. Scott, V. Chivukula, J. Lukasik, S. R. Farrah and J. B. Rose, *Appl. Environ. Microbiol.*, 2005, **71**, 3163–3170.
46 I. R. Cooper, H. D. Taylor and G. W. Hanlon, *J. Appl. Microbiol.*, 2007, **102**, 1293–1299.
47 B. W. Webb and F. Nobilis, *Hydrol. Processes*, 1997, **11**, 137–147.
48 M. N. B. Momba, A. N. Osode and M. Sibewu, *Water SA*, 2006, **32**, 687–692.
49 J. R. Stewart, R. J. Gast, R. S. Fujioka, H. M. Solo-Gabriele, J. S. Meschke, L. A. Amaral-Zettler, E. del Castillo, M. F. Polz, T. K. Collier, M. S. Strom, C. D. Sinigalliano, P. D. R. Moeller and A. F. Holland, *Environ. Health*, 2008, **7**(Suppl. 2), S3.
50 K. T. Goetz, D. J. Rugh, A. J. Read and R. C. Hobbs, *Mar. Ecol.: Prog. Ser.*, 2007, **330**, 247–256.
51 J. P. Schroeder, J. G. Wallace, M. B. Cates, S. B. Greco and P. W. B. Moore, *J. Wildl. Dis.*, 1985, **21**, 437–438.
52 J. S. Yoder, C. Harral and M. J. Beach, *MMWR*, 2010, **59**, 15–25.